



NanoDrop 8000 Spectrophotometer

V1.1 User's Manual

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Revised 3/08

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1. Overview

Instrument Description

The Thermo Scientific NanoDrop™ 8000 Spectrophotometer is a full-spectrum (220-750nm) instrument that measures 8 individual 1 ul samples with high accuracy and reproducibility. It utilizes the same patented sample retention technology utilized on the NanoDrop 1000 Spectrophotometer and the NanoDrop 3300 Fluorospectrometer. The surface retention system holds the sample in place eliminating the need for cumbersome cuvettes and other sample containment devices. Clean-up is accomplished in seconds. In addition, the NanoDrop 8000 has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

Operation

Up to eight 1 ul samples are pipetted onto the sample pedestal using a low volume multi-channel pipettor.

Each position is actually the end of a fiber optic cable (the receiving fibers). A second set of fiber optic cables (the source fibers) are brought into contact with the liquid samples causing the liquid to bridge the gaps between the fiber optic ends. The pathlengths are automatically controlled to 1mm and 0.2 mm paths. Readings are acquired through sequential measurement across the 8 positions. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array is used to analyze the light that passes through the samples. The instrument is controlled by PC based software, and the data is logged in an archive file on the PC.

The NanoDrop 8000 is designed only for indoor use under the following conditions:

- Temperature: 40-100° F (4.4 - 37.8° C)
- Humidity: 10-90%

Applications

UV/VIS spectrophotometry is simple for samples as small as 1 ul using the NanoDrop 8000 Spectrophotometer. The small sample requirement and ease of use make the NanoDrop 8000 Spectrophotometer ideally suited for measuring:

- Nucleic acid concentration and purity of nucleic acid samples up to 3700 ng/ul (dsDNA) without dilution
- Fluorescent dye labeling density of nucleic acid microarray samples
- Purified protein analysis (A280 nm) up to 100 mg/ml (BSA)
- Expanded spectrum measurement and quantitation of fluorescent dye labeled proteins, conjugates, and metalloproteins
- Bradford Assay analysis of protein
- BCA Assay analysis of protein
- Lowry Assay analysis of protein
- Cell density measurements
- General UV-Vis spectrophotometry

Patents

The sample retention technology used in the NanoDrop 8000 is covered under US patents 6,628,382 and 6,809,826. Other patents are pending.

2. Initial Set Up

Computer Requirements

The operating software will only run on an IBM compatible PC meeting the below criteria. No Mac versions of the software are currently available.

- Microsoft Windows XP or 2000 operating system.
Windows Vista has also been tested successfully with the software.
- **The operating software is not compatible with Windows NT, 95, 98 or ME.**
- 800 MHz or higher processor
- CD ROM drive
- 128 MB or more of RAM
- 100 MB of free hard disk space
- Open USB port (the instrument can only be connected via the USB port)
- Microsoft Excel or other spreadsheet program to manipulate archived data (optional)

Software Installation

WARNING: The system software must be loaded onto the PC before the USB cable is connected. Administrator access on the PC is required to install the software.

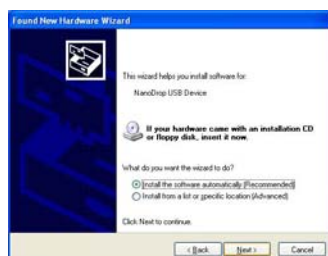
When attaching the USB cable, please wait at least 30 seconds for the USB devices and internal drivers to be installed and recognized.

To properly install the operating software:

1. Close all programs and make sure that the USB cable is unplugged.
2. Insert the operating software CD in the CD drive of the PC. The software installation menu should appear automatically. If software menu does not appear, choose 'My Computer' to view the contents of the CD. Double click on the file named 'nd-8000...install.exe'.
3. After software installation, connect the USB cable and the Found New Hardware Wizard should start as shown below. Windows XP SP2 operating system will ask to allow it to search the internet for the proper software as shown - Select 'No, not this time'. Follow the prompts for automatic installation of the software.

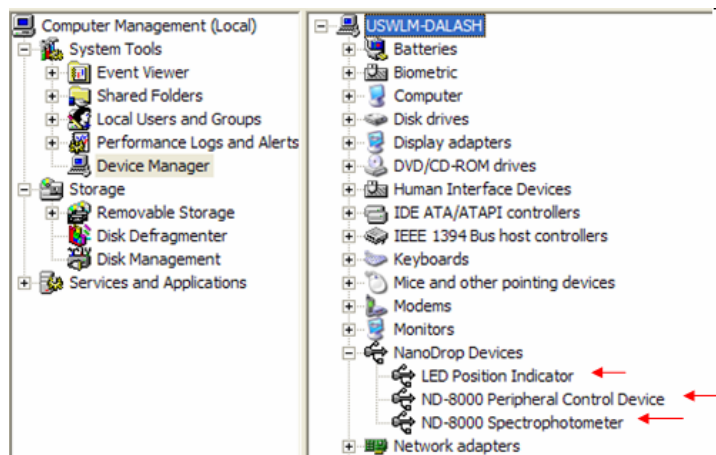


Intro Page: Windows XP- SP2



All Windows Operating Systems

4. Installation will require two cycles through the Found New Hardware Wizard, once for the NanoDrop 8000 Spectrophotometer and once for two devices internal to the instrument. For the NanoDrop 8000 to operate successfully a **total of three USB devices** need to install although only two cycles through the hardware wizard will be observed. To confirm installation, view the Windows Device Manager as shown below:



Your NanoDrop 8000 Spectrophotometer should now be ready for operation. If the software does not start properly, refer to the Troubleshooting section for possible solutions.

Configuring the System Font

The software is designed to look best with the MS Sans Serif font, 8 point. To check that the system font is set to the proper selection:

1. Open the 'Display Properties' by right clicking on the desktop and select *Properties* → *Appearance*. (Additional step for Windows XP: click on the 'Advanced' button).
2. From 'item' list select 'icon'.
3. Select the 'MS Sans Serif (western)' font and select '8 point' size.
4. Click OK.

Choosing an alternative font may result in some text being truncated in the operating software window.

Software Upgrades

Periodic upgrades are made to the operating software and are available for download. See our [website](#) for the latest available software version.

USB Flash Drive Port

Any standard PC USB flash drive may be used for exporting data. Note: When using the User Preferences Module to set-up a default automatic "Export Report" destination, keep in mind that the flash drive may not always be assigned the same removable device designation.

Cable Connections

To make measurements with the instrument, connect the USB cable to instrument and the PC, plug in the 12V power supply and connect to the power input at the back of the instrument.

Note: The NanoDrop 8000 Spectrophotometer is supplied with a 12V power supply. Use only the power supply provided with the kit. The unit also comes with a grounded power cord. Plug this cord **ONLY** into a properly grounded outlet. Use of the instrument in a manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply.

The power supply can remain plugged into the NanoDrop 8000 Spectrophotometer while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~3 W and the flashlamp is not energized. Also, the instrument does not utilize a power switch or give a visual indication of the operability of the 12V power supply.

Note: It is recommended that the instrument not be positioned in a way that makes it difficult to unplug the power supply from the unit or the wall.

Registering Your Instrument

Please register your product! We periodically update our software and add new features free of charge. We would like to keep our user list updated so that we may alert you to these updates and all information supplied is completely confidential. You can register your instrument on our [website](#).

Lock Attachment Port

The NanoDrop 8000 is equipped with a lock slot that enables use of a standard locking cable typically used to secure a laptop PC.

3. General Operation

The Sample Retention System

The main steps for using the sample retention system are listed below:



1. With the sampling arm open, position the pipettor using the guide as shown. Dispense the samples onto the lower measurement pedestal ensuring the samples “touch-off” (contact) the lower pedestal. Carefully withdraw the pipettor before releasing the pipettor’s dispensing mechanism. Visually verify all samples are correctly transferred to their respective pedestals.



2. Close the sampling arm and initiate a spectral measurement using the operating software on the PC. The sample columns are automatically drawn between the upper and lower measurement pedestals and the spectral measurement made.

3. When the measurement is complete, open the sampling arm and wipe the samples from both the upper and lower pedestals using a soft laboratory wipe.



Cleaning the Sample Retention System

Wiping the sample from both the upper and lower pedestals (as shown above) upon completion of each sample measurement is usually sufficient to prevent sample carryover and avoid residue buildup. Although generally not necessary, 2 ul water aliquots can be used to clean the measurement surfaces after particularly high concentration samples to ensure no residual sample is retained on either pedestal. After measuring a large number of samples, however, it is recommended that the areas around the upper and lower pedestals be cleaned thoroughly. A final cleaning of all surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do **not** use a squirt bottle to apply de-ionized water.

Decontamination of Measurement Pedestals

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. The metal fiber optic fittings are made from 303 stainless steel and are resistant to most common laboratory solvents (see “Solvent Compatibility” appendix). A final cleaning of all surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do **not** use a squirt bottle to apply bleach or de-ionized water.

Rapid Reconditioning of the Sample Retention System

The Bradford reagent as well as other buffers containing surfactants may “un-condition” the measurement pedestal surfaces so that the liquid column does not form well with 1ul samples. Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

Sample Size Requirements

Although sample size is not critical, it is essential that the liquid column be formed so that the gap between the upper and lower measurement pedestals is bridged with sample. Note: It is not necessary to have liquid on all 8 positions to make a measurement.

Field experience indicates that the following volumes are sufficient to ensure reproducibility:

- Aqueous solutions of nucleic acids: 1 ul
- Purified protein: 2 ul
- Bradford, BCA or Lowry assay: 2 ul
- Microbial cell suspensions: 1-2 ul

It is best to use a precision pipettor (0-2 ul) with precision tips to ensure that sufficient sample (1-2 ul) is used. Lower precision pipettors (0-10 ul and larger) are not as good at delivering 1 ul volumes to the measurement pedestal. If you are unsure about your sample surface tension characteristics or pipettor accuracy, a 2 ul sample is recommended.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Sample Carryover

Prevention of sample being retained on the NanoDrop 8000 Spectrophotometer's measurement pedestals is easily addressed. Simple wiping of the upper and lower measurement pedestal with a dry laboratory wipe is highly effective in eliminating carryover for samples differing in concentration by as much as three orders of magnitude (see our [website](#) for NanoDrop 1000 carryover data).

Sample Homogeneity

Sampling from non-homogeneous solutions – particularly when using small volumes – can cause significant deviations in the data generated using all measurement technologies including spectrophotometry. Genomic DNA, lambda DNA and viscous solutions of other highly concentrated nucleic acids are common examples known to the molecular biologist. Proteins are subject to denaturation, precipitation, and aggregation and therefore may require special handling to ensure sample homogeneity.

Effect of Evaporation and Solvents

Evaporation of the sample during the measurement cycle usually has just a minimal effect on absorbance readings and may result in a 1-2% increase in sample concentration. This can be observed in the field by measuring the same sample successively over time. Highly volatile solvents, such as hexane, will likely evaporate before the measurement can be completed. Less volatile solvents such as DMSO can be used successfully.

To minimize the effects of evaporation, it is recommended that an 8-channel, low volume pipettor be used to simultaneously dispense samples onto the measurement pedestals.

Sample Recovery

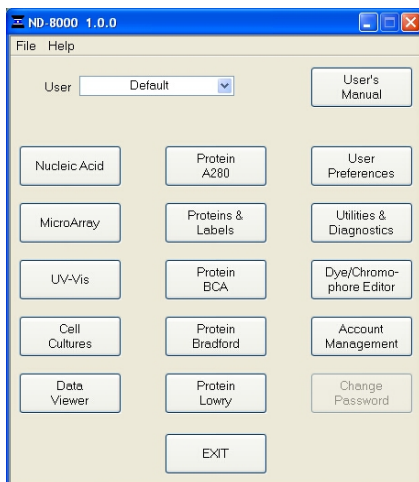
One of the advantages of the sample retention system is that samples can be recovered from the upper and lower measurement pedestals by extraction with a pipette.

Software Architecture and Features

Main Menu

With the sampling arm in the down position, start the operating software by selecting the following path:

Start → Programs → NanoDrop → ND-8000 (version)



Application Modules

The operating software has been tailored to meet the life scientist's needs. It includes the following application modules:

- **Nucleic Acid** – concentration and purity of nucleic acid
- **MicroArray** – dye incorporation concentration and purity of nucleic acid
- **UV-Vis** – general UV-Vis measurements
- **Cell Cultures** – absorbance (light scattering) measurement of suspended microbial cells
- **Protein A280** – concentration and purity of purified protein
- **Proteins & Labels** – concentration of dye-labeled proteins, conjugates, and metalloproteins
- **Protein BCA** – protein concentration using the BCA assay
- **Protein Bradford** – protein concentration using the Bradford assay
- **Protein Lowry** – protein concentration using the Modified Lowry assay

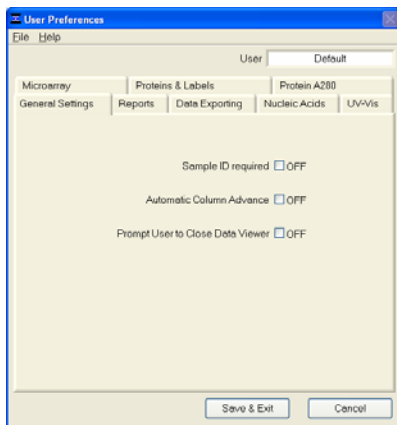
User Preferences

Each user has the option to configure a number of settings in the various application modules. The user preferences options for each application module are self explanatory and include options applicable for that module.

Some key features include:

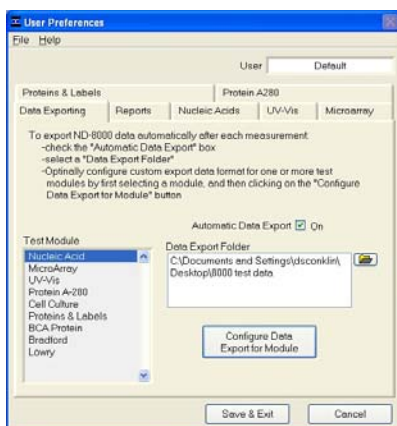
The General Settings tab allows the user to either select or deselect as default settings the options of:

- Requiring sample ID's before a measurement is taken
- Auto advancement to the next set of samples (i.e. move to the next column on the 96 well visual)
- Requiring the user to confirm that they want the Data Viewer to close when closing out of an acquisition module

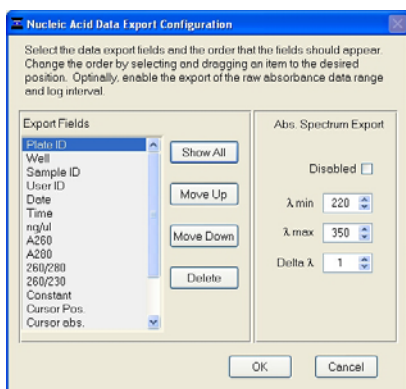


Data Exporting

In addition to the primary data storage of archive files at *c:\IND-8000 Data*, users may elect to export their data to an additional location. This option can be chosen under the 'Data Exporting' tab by selecting the 'Automatic Data Export' box and then choosing the file path by clicking on the file folder icon under 'Data Export Folder'. The archived files may be opened with Excel by using the right click option of the mouse to open the .nd8 file. Note: It is important to save the file with a new name before making changes with Excel.



Selecting the 'Configure Data Export for Module' button will bring up the following pop-up box:



This box is used to select the particular data columns of interest when exporting data. Save the designated path by clicking on the Save & Exit button before exiting the User Preferences module.

Auto Reporting

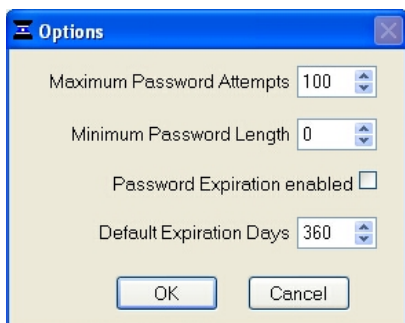
Users may choose to select the 'Auto Reporting' option for any of the application modules. The auto reporting option allows data to automatically be saved to the report for all samples. Users may choose this option under the Reports tab by selecting the corresponding box next to the modules listed under Auto Reporting. Save the auto reporting functions by clicking on the Save & Exit button before exiting the User Preferences window.

Note: User preferences are stored in a '.log' file. When upgrading to a newer version of the software, this file should be preserved. If the user preferences do not appear correctly after upgrading to a new software version, the .log file should be manually copied to the proper directory. This file contains the User ID & password for all accounts and is readable only by the software. It can be found in the *c:\IND-8000 Data\log files* folder. It is strongly recommended that each time a new user account is added or a password is changed, the administrator make a **copy** of the updated file and store it in the *c:\IND-8000 Data\log files* folder. If the administrator's account becomes locked, the up-to-date copy can be renamed and used as the password.log file.

Utilities and Diagnostics

This module is used to help troubleshoot operational problems with the instrument. For more information on using this module, refer to the Troubleshooting section of this manual.

Note: The administrator, using the 'Options' or the 'Modify User' entries in the 'Account Management' module, establishes whether individual user passwords will expire and, if so, after how many days.



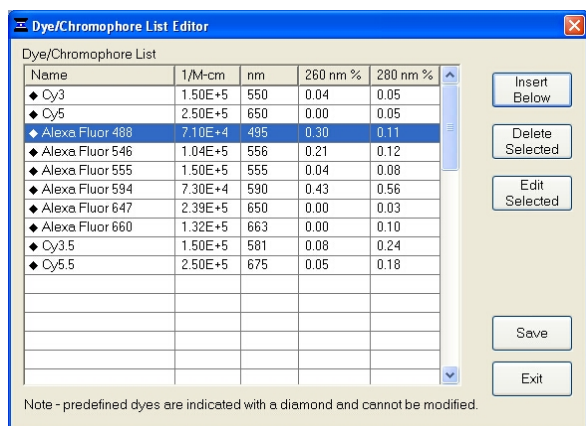
Passwords.log file

This file contains the User ID & password for all accounts and is readable only by the software. It can be found in the *c:\IND-8000 Data\log files* folder. It is strongly recommended that each time a new user account is added or a password is changed, the administrator make a **copy** of the updated file and store it in the *c:\IND-8000 Data\log files* folder. If the administrator's account becomes locked, the up-to-date copy can be renamed and used as the password.log file.

Dye/Chromophore Editor

The Dye/Chromophore Editor gives the user the ability to add additional dyes or chromophores to the list of predefined fluorescent dyes available for use with the MicroArray and Proteins & Labels modules. Predefined dye methods are indicated by a diamond and cannot be modified.

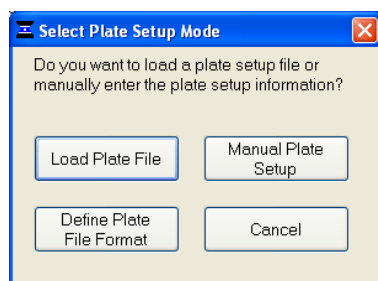
Absorbance contribution at 260nm and 280nm from the respective dye can be corrected by entering the appropriate decimal correction in the respective field when adding a new dye to the list. Please refer to the dye manufacturer for the appropriate correction factors for user-entered dyes.



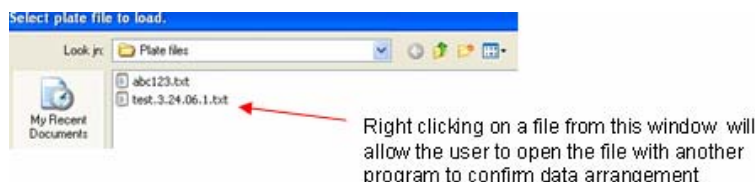
4. Plate Set-up

Selecting a Plate Setup

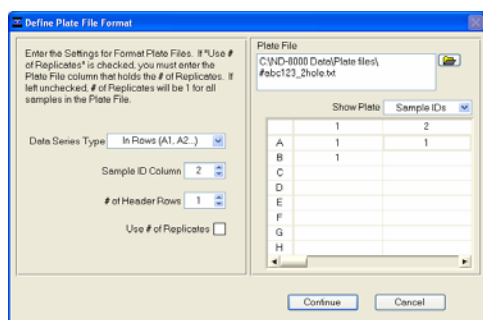
After the instrument has completed the initialization process the following window will automatically display the options for plate setup:



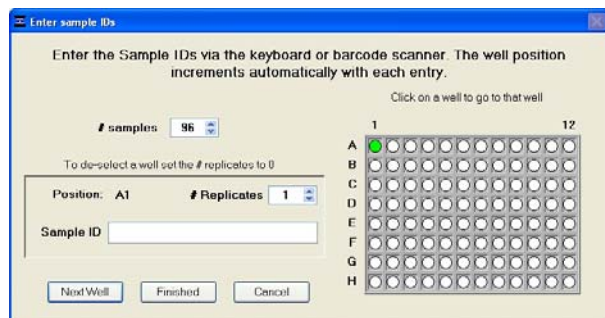
- Load Plate File** - This option opens the Plate file folder enabling the user to load a pre-configured plate file. Plate files may be created using either Notepad or Excel. When creating a file, enter the sample names in column 1 and the number of replicates desired for each sample in column 2. The file should be saved as a .txt file at *C:\ND-8000 data\ Plate Files*. In addition, individual bar-coded samples may be entered (scanned) into the text file. **Note: If a plate file format has been defined (see below), that format will be retained and applied to subsequent plates until the user selects a different format. For this reason, when loading a pre-configured plate file, it is crucial for a user to know whether the data is arranged by rows or in columns to ensure correct sample identification on the report. Right clicking on the specific file from the “Select plate file to load” window will allow the user to open the file with a program such as Excel or Notepad to confirm data arrangement.**



- Define Plate File Format** - This option allows the user to define the data format of a saved plate file. The user is first prompted to select a pre-existing plate file from the Plate file folder. Once a plate file has been selected the Define Plate File Format window will appear. This window enables the user to define the data series type (rows or columns), which column contains the sample ID, whether headers are defined in the data set and the number of replicates for each sample. Selecting Continue after defining the format will load the plate file. **Note: Once a plate file format has been defined, that format will be retained and applied to subsequent plates until the user selects a different format. For this reason, it is crucial for a user to know whether the data is arranged by rows or in columns to ensure correct sample identification on the report. Right clicking on the specific file from the “Select plate file to load” window will allow the user to open the file with a program such as Excel or Notepad to confirm data arrangement.**



- Manual Plate Setup** - This option opens the Enter Sample IDs window enabling the user to manually enter samples IDs and number of replicates for each sample to be tested as prompted by the following screen:

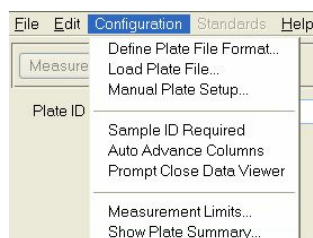


To move to the next well in a column, enter the sample name and then click on the Next Well button. To select a well in another column, highlight the well of interest, enter in the sample name and hit the keyboard 'Enter' button.

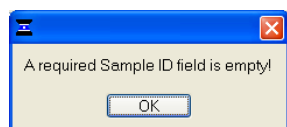
- **Cancel** - Closes the window without making any changes.

Configuration

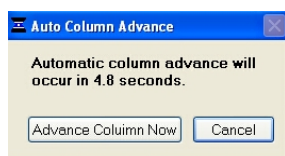
If the user cancels the Plate Setup Mode without loading a plate file, the above operations may also be accessed from the Configuration drop down on the main acquisition page. From the Configuration drop down the user has the following options:



- **Sample ID Required** - If selected, the sample ID field must be populated for each sample tested. Wells requiring a sample ID will appear red in the sample status color code (see below for description of this feature) and the following message will appear when the user attempts to make a sample measurement.



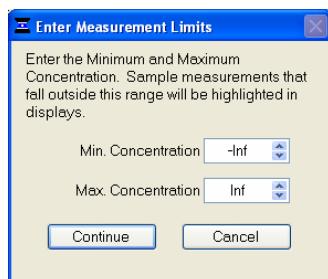
- **Auto Advance Columns** - If selected, there is a 5 second delay before the displayed spectra clear and the status code and Sample Position Illuminator (see below for description of these features) advance to the next column for sampling. This gives the user the option of canceling auto advance for that column. Auto advance will restart when the next column is measured. This feature can be set as a default function under the User Preferences application.



Note: All data is automatically archived and is accessible through the Data Viewer.

- **Prompt Close Data Viewer** - If selected, the user will be given the option of closing the Data Viewer when exiting the software module.

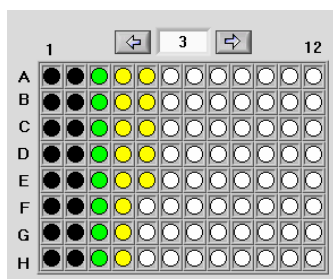
- **Measurement Limits** - Allows the user to set minimum and maximum concentration limits for samples to be measured. Additional information about setting measurement limits is included in each application module section.



- **Show Plate Summary** - Allows the user to review the results of the plate at any time during the measurement session. Once the entire plate has been read, this feature will allow the user the option of marking samples of interest for repeat testing

Sample Status Color Code

The 96 well plate schematic allows the user to monitor the position of the samples being measured. Black wells indicate that samples in the corresponding wells have been measured. Green wells indicate which positions are currently being measured while yellow represents well positions that are expected to be measured. Pre-loading a plate file or manually entering in sample names will activate the respective wells. To select additional wells, use the Configuration drop down box and select Manual Plate Set-up. If Sample ID Required has been selected from the configuration drop down, any wells that do not have an ID assigned will appear red and a sample ID must be assigned before measurement can begin.



Sample Position Illuminator

This lighted guide allows the user to visualize the row of a standard 96-well micro titer plate that is to be sampled for measurement. The Sample Position Illuminator corresponds to the sample status color code displayed on the software screen and the pattern of illumination is determined by plate configuration at set up. If measurement limits have been defined, wells that are out of the defined range will flash on the Sample Position Illuminator immediately after measurement. These positions will continue flashing until measurement of the current plate sample set is complete, the results have been reviewed and the Plate Results Summary window is closed.



Plate Review

The Plate Results Summary window will automatically appear if the auto advance column feature has been selected and measurement of the plate sample set is complete. Selecting Show Plate Summary from the configuration drop down menu will also open this window. From this window, sample wells of interest can be marked for repeat. If measurement

limits were defined, sample measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator and the concentrations will appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Plate Results Summary

Plate ID: 425 ng- μ l

Plate Results (ng/ μ l)

	1	2	3	4	5	6	7	8	9	10	11	12
A	458.2	458.6	459.6	460.7	460.3	460.0	460.3	458.9	458.0	459.5	458.6	458.8
B	458.5	460.9	460.9	461.2	459.6	460.6	465.9	461.0	459.8	461.2	461.1	461.6
C	456.9	458.8	456.5	457.7	456.9	457.8	458.9	458.3	458.2	458.4	461.6	457.7
D	459.0	459.2	458.7	458.6	459.0	460.1	460.2	459.5	461.1	461.3	459.8	460.0
E	457.0	459.5	457.1	458.5	458.3	458.6	464.2	459.3	460.5	463.2	459.7	459.6
F	467.5	458.6	458.6	458.9	458.0	459.3	457.5	459.3	458.6	459.5	459.6	459.6
G	461.4	462.2	460.3	461.6	459.8	458.6	464.8	461.6	462.3	461.3	461.7	460.8
H	458.6	459.9	456.9	458.6	457.4	459.0	460.8	458.7	458.5	460.5	460.6	460.2

Cell Color Code:
 White: inside limits
 Red: outside limits
 Light green: inside limits & selected
 Dark green: outside limits & selected

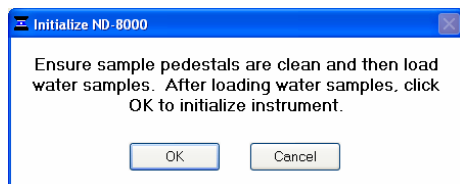
Click on any cell to select/de-select that cell for repeat measurements.

Repeat Selected Close Window

5. Common Module Functions

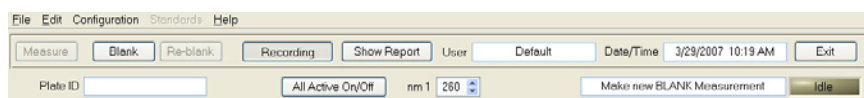
Module Startup

When a software module is opened, the first message seen will indicate that the instrument motors are initializing. After that quick step is complete, the next message will appear:



For best results, **ensure measurement pedestal surfaces are clean** and load 2 ul of water onto **each** lower measurement pedestal. Lower the arm and click OK. The message 'Please wait - Initializing Spectrometer' will then appear. When this message disappears, the instrument will be ready for use. All data taken will automatically be logged in the appropriate archive file.

Module Functions



Measure (F1)

Each time a software module is opened (initiated), the Measure button is inactive as noted by its "grayed-out" appearance. A blank must first be measured before the Measure button will become active.

The Measure button is used to initiate the measurement sequence for all samples (non-blanks). It is activated by depressing the F1 key or clicking the Measure button. The Sample Position Illuminator allows the user to visualize the row of a standard 96-well micro titer plate that is to be sampled for measurement and corresponds to the sample status color coded guide on the screen. See the section on plate set up for more information about the Sample Position Illuminator. The entire measurement cycle takes approximately 20 seconds (less time if fewer than 8 positions are used.)

Blank (F2)

Before making a sample measurement, a blank must be measured and stored. All eight positions are blanked with each blanking command. Note: The software initiates each blank and measurement cycle on the first position to be read. The user will, therefore, hear one less position increment than expected. After making an initial blank measurement, a straight line will appear on the individual graphs. Subsequent blanks will clear any sample spectrum and again display straight lines.

Blanking Cycle

For the most consistent results, it is best to begin any measurement session with a blanking cycle.

- Open the application software module.
- Load an aliquot of the blank (the same buffer, or solvent the unknown samples are in) onto each of the lower measurement pedestals and then lower the sampling arm into the 'down' position.
- Click on the Blank button. When the measurement is complete, wipe the buffer from all pedestals.
- Select "All Active On" and analyze a fresh aliquot of the blanking solution on all pedestals using the 'Measure' button (F1). The result should be 8 spectra with relatively flat baselines near zero.
- Wipe the blank from both measurement pedestal surfaces with a laboratory wipe and repeat the process until the spectrum varies no more than 0.005 A (1mm path).

Reload the plate list file before measuring samples if necessary.

See "Blanking and Absorbance Calculations" in the appendix for more information on blanking and absorbance calculations.

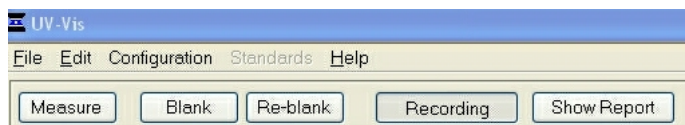
Re-blank (F3)

The Re-blanking option establishes a new reference (blank) that is used for the absorbance calculations of subsequent samples. The Re-blank is only applied to the specific samples selected and re-calculates the concentration for those samples respectively.

See the “Blanking and Absorbance Calculations” appendix for more information on absorbance calculations.

Start Report / Recording

All data is automatically archived. The user can log measurement results in a active report table as the data is accumulating by using the Start Report / Recording feature. The default setting has the Recording feature activated for all modules. If ‘Start Report’ is displayed, the accumulating data will still be archived but will not be shown in the active report.



Show Report

Selecting this button will bring up the Report page, which is part of the integrated Data Viewer software. A full description of the features and options for the Report page can be found in the section on the Archived Data and Data Viewer.

Plate ID

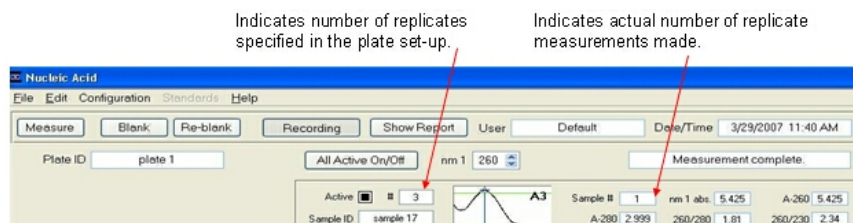
The Plate ID will be automatically populated if a plate file is imported. Alternately- the user may manually type in a Plate ID or other identifying descriptor. The Sample Position Illuminator allows the user to visualize the row of a standard 96-well micro titer plate that is to be sampled for measurement. This lighted guide corresponds to the sample status color code displayed on the software screen and the pattern of illumination is determined by plate configuration at set up.

Status

The Status button will turn green during a measurement cycle.

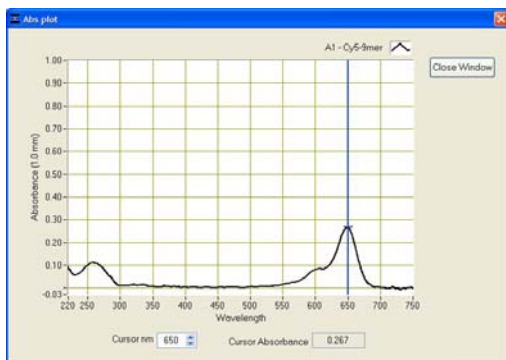
and Sample

The # indicator to the **left** of the spectra indicates the number of replicates originally called for when sample names were entered in manually or by loading a plate file. The Sample # located to the **right** of the spectrum is activated when a sample measurement is being recorded. It indicates the replicate number of the last sample processed for a particular well and increments with each successive measurement.



Expanded Sample Spectrum View

The user may display an expanded view of a single spectrum by clicking on the spectrum of interest. This view will display one movable cursor with the respective nm and absorbance reported in the boxes at the bottom of the screen. Note: Data is archived as the measurement is made. Changing the cursor position after the data is archived will not change the data files. Moving the cursor via the expanded view will not change the selected wavelength positions on the main acquisition page.



Print Window

A Print dialogue can be initiated from the File pull-down menu or by typing 'Ctrl+P'.

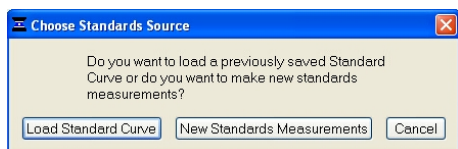
Sample ID

The Sample ID is highlighted for overtyping or barcode scanning. The user may input a sample ID that will be used to identify the measurement in a report print and in the archived data file. The sample ID entry is "key focused", meaning it is the default selection on the screen and should have a flashing text cursor when the instrument is waiting to make a new measurement.

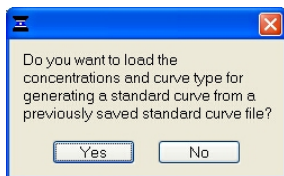
Standard Curves

A standard curve is required every time the BCA, Lowry, and Bradford assay are run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines and generate fresh standard curves for each assay. Both single and multi-point standard curve generation is incorporated into the software. A standard curve can be developed using a reference (assay reagent only – no protein) and a single replicate of one standard. There is no set order in which standards must be run. The multi-point standard curve generator allows a maximum of 5 replicates for each of 7 different standards.

The following box will appear after the module initialization is complete:



The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:



Measurements Table		Double Click on any row to change the concentration or delete replicates						
	Standard	mg/ml	Ave Abs	Abs. 1	Abs. 2	Abs. 3	Abs. 4	Abs. 5
Active	A-Reference	0.000						
Active	B-Standard 1	1.000						
Active	C-Standard 2	2.000						
Active	D-Standard 3	3.000						
Active	E-Standard 4	4.500						
Active	F-Standard 5	6.000						
Inactive	G-Standard 6							
Inactive	H-Standard 7							

Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards.

The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve. See the respective protein assay section for additional details.

User's Manual

The User's Manual is accessible from the Main Menu and from the Help menu in all of the application modules. It can also be accessed by selecting *Start* → *Programs* → *NanoDrop* → *ND-8000 (version)*.

Saving Current Screen as .JPG Image

The current screen can be saved as a .JPG image file by selecting 'Save Window' from the File pull down menu.

Exit

This command closes all application modules and supporting options. After clicking the 'Exit' button, the user has 10 seconds to cancel the exit command. If no action is taken within 10 seconds, the exit command is carried out. Note: All measurement data is automatically saved to an archive file and requires no user action.

Escape Key (ESC)

The escape key is set to exit out of all screens. Hitting the escape key twice will log the user out of an application module.

6. Nucleic Acids

Nucleic acid samples can be readily checked for concentration and quality using the NanoDrop 8000 Spectrophotometer. To measure nucleic acid samples, select the 'Nucleic Acid' application module on the Main Menu.

Sample Volume Requirements

Field experience has indicated that 1ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous nucleic acid samples. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

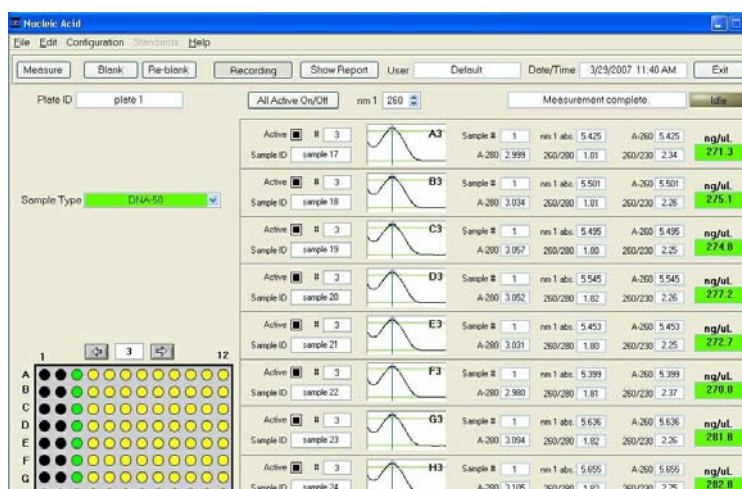
Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer will accurately measure dsDNA samples up to 3700 ng/ul without dilution. To do this, the instrument detects the high concentration and automatically utilizes the 0.2mm pathlength to calculate the absorbance. The table below lists the concentration range and typical reproducibility for nucleic acid measurements on the NanoDrop 8000:

Detection Limit (ng/ul)	Approx. Upper Limit (ng/ul)	Typical Reproducibility (minimum 96 replicates) (SD= ng/ul; CV= %)
2.5	3700 ng/ul (dsDNA) 3000 (RNA) 2400 (ssDNA)	sample range 2.5-100 ng/ul: ± 2.5 ng/ul sample range >100 ng/ul: $\pm 2.5\%$

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for nucleic acid measurements as defined by the absorbance at 260 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Nucleic acid measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **Sample Type:** used to select the (color-keyed) type of nucleic acid being measured. The user can select 'DNA-50' for dsDNA, 'RNA-40' for RNA, 'ssDNA-33' for single-stranded DNA, or 'Other' for other nucleic acids. The default is DNA-50. If 'Other' is selected, the user can select an analysis constant between 15-150. When navigating amongst the three general sample types within the Nucleic Acids module, the last constant value entered within the 'Constant'

sample type will be retained. See the "Concentration Calculation (Beer's Law)" Appendix for more details on this calculation.

- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding 10 mm equivalent normalized absorbance at the respective wavelength absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.
- **A260:** absorbance of the sample at 260 nm represented as if measured with a conventional 10 mm path. Note: This is 10X the absorbance actually measured using the 1 mm path length and 50X the absorbance actually measured using the 0.2 mm path length.
- **A280:** sample absorbance at 280 nm is represented as if measured with a conventional 10 mm path. Note: This is 10X the absorbance actually measured using the 1 mm path length and 50X the absorbance actually measured using the 0.2 mm path length.
- **260/280:** ratio of sample absorbance at 260 nm and 280 nm. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. See "260/280 Ratio" in the Troubleshooting section for more details on factors that can affect this ratio.
- **260/230:** ratio of sample absorbance at 260 nm and 230 nm. This is a secondary measure of nucleic acid purity. The 260/230 values for "pure" nucleic acid are often higher than the respective 260/280 values. They are commonly in the range of 1.8-2.2. If the ratio is appreciably lower, this may indicate the presence of co-purified contaminants.
- **ng/ul:** sample concentration in ng/ul based on absorbance at 260 nm and the selected analysis constant. See the "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

Spectrum Normalization

The baseline is automatically set to the absorbance value of the sample at 340 nm, which should be very nearly zero absorbance. All spectra are referenced off of this zero.

7. MicroArray

The capability to pre-select viable fluorescent-tagged hybridization probes for gene expression in micro-arrays can eliminate potentially flawed samples and improve research effectiveness. The NanoDrop 8000 Spectrophotometer measures the absorbance of the fluorescent dye, allowing detection at dye concentrations as low as 0.2 picomoles per microliter.

Fluorescent Dye Selection

There are currently ten fluorescent dyes that are hard-coded for use with the MicroArray module (see table below). Users can also enter & save fluorescent dyes not coded within the NanoDrop 8000 software using the 'Dye/Chromophore Editor' button found in the main menu.

Dyes can be selected using the scroll arrows or by highlighting the Dye box. The respective absorbance wavelength, extinction coefficient, and 260 nm and 280 nm % corrections will be automatically utilized for measurement and concentration calculation. The default setting is Cy3.

Note: Please refer to the dye manufacturer for the appropriate correction factors for user entered dyes.

Name	1/M-cm	nm	260 nm %	280 nm %
◆ Cy3	1.50E+5	550	0.04	0.05
◆ Cy5	2.50E+5	650	0.00	0.05
◆ Alexa Fluor 488	7.10E+4	495	0.30	0.11
◆ Alexa Fluor 546	1.04E+5	556	0.21	0.12
◆ Alexa Fluor 555	1.50E+5	555	0.04	0.08
◆ Alexa Fluor 594	7.30E+4	590	0.43	0.56
◆ Alexa Fluor 647	2.39E+5	650	0.00	0.03
◆ Alexa Fluor 660	1.32E+5	663	0.00	0.10
◆ Cy3.5	1.50E+5	581	0.08	0.24
◆ Cy5.5	2.50E+5	675	0.05	0.18

Note - predefined dyes are indicated with a diamond and cannot be modified.

Sample Volume Requirements

Field experience has indicated that 1 ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous nucleic acid samples containing incorporated fluorescent dyes. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

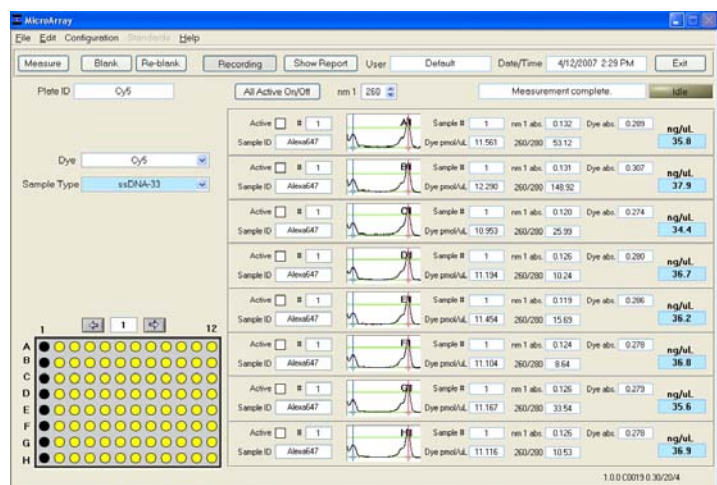
Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer will accurately measure fluorescent-dye and nucleic acid concentrations up to 100 pmols/ul (Cy3) and 750 ng/ul (DNA) respectively without dilution. A table of sample concentration ranges is listed below.

Sample Type	Detection Limit (pmol/ul)	Approx. Upper Limit (pmol/ul)	Typical Reproducibility (minimum 48 replicates) (SD= pmol/ul; CV= %)
Cy3, Cy3.5, Alexa Fluor 555 and Alexa Fluor 660	0.25	100	sample range 0.25-4 pmol/ul: $\pm$ 0.25 sample range >4.0 pmol/ul: $\pm$ 2.5%
Cy5, Cy5.5 and Alexa Fluor 647	0.17	60	sample range 0.17-2.4 pmol/ul: $\pm$ 0.17 sample range >2.4 pmol/ul: $\pm$ 2.5%
Alexa Fluor 488 and Alexa Fluor 594	0.50	215	sample range 0.50-8.0 pmol/ul: $\pm$ 0.50 sample range >8.0 pmol/ul: $\pm$ 2.5%
Alexa Fluor 546	0.42	145	sample range 0.42-6.0 pmol/ul: $\pm$ 0.42 sample range >6.0 pmol/ul: $\pm$ 2.5%

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for the nucleic acid component as defined by the absorbance at 260 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Nucleic acid measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **Sample Type:** used to select the (color-keyed) type of nucleic acid being measured. The user can select 'DNA-50' for dsDNA, 'RNA-40' for RNA, ssDNA-33' for single-stranded DNA, or 'Other' for other nucleic acids. The default is ssDNA-33. If 'Other' is selected, the user can select an analysis constant between 15-150. When navigating amongst the three (3) general sample types within the MicroArray module, the last value of the constant entered within the Constant Sample Type will be retained. See "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.
- **nm 1 and nm 1 abs:** current values of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.
- **Dye:** user selected dye.
- **Dye pmol/ul:** concentration based upon selected dye's extinction coefficient. See "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.
- **ng/ul:** concentration of nucleic acids in the sample calculated using the absorbance at 260 nm minus the absorbance at 340 nm (i.e. normalized at 340 nm). See "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.
- **260/280:** ratio of sample absorbance at 260 nm and 280 nm. The ratio of absorbance at 260 and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. See "260/280 Ratio" in the Troubleshooting section for more details on factors that can affect this ratio.

Baseline Calculation & Normalization

The software normalizes the visual spectrum display for all readings at 750 nm and will automatically calculate a baseline between 400 and 750 nm for dye concentration calculations.

8. UV-VIS

The 'UV/VIS Absorbance' module allows the NanoDrop 8000 Spectrophotometer to function as a conventional spectrophotometer. Sample absorbance is displayed on the screen from 220 nm to 750 nm and cursors permit the measurement of individual peaks.

Sample Volume Requirements

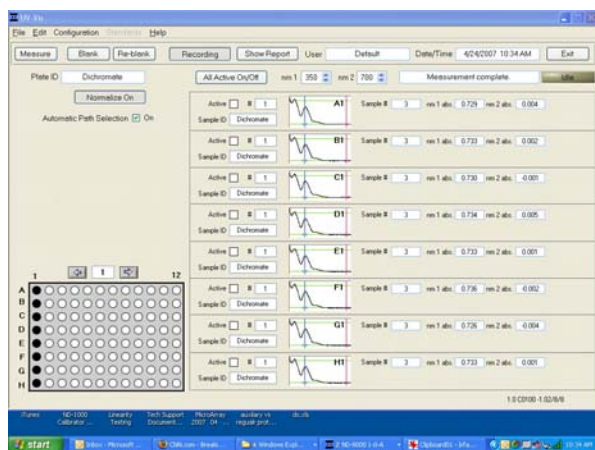
Field experience has indicated that 1 ul samples are sufficient to ensure accurate and reproducible results when measuring most aqueous samples. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer is configured to measure absorbance up to the 1 mm pathlength equivalent of 7.5 A when utilizing the 0.2 mm path via the automatic path selection feature. By selecting Measurement Limits from the configuration drop down menu, minimum and maximum absorbance limits can be set for the shorter of the two user selectable wavelengths. These limits cannot be set as a default and must be defined each time the application module is opened. Sample absorbances that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The absorbances will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing

Unique Screen Features



- **Automatic Path Selection:** a user-selectable feature unique to this module. If selected, the software will automatically switch from using the 1.0 mm pathlength to the 0.2 mm path when the absorbance value first reaches 1.25 for EITHER of the two wavelengths indicated with the two cursors.

Note: It is important to pre-select a cursor position for the wavelength of interest before the measurement is taken. If the cursors are set for wavelengths with minimal absorbance, the 0.2 mm pathlength will not be utilized.

Note: When the 0.2 mm pathlength is utilized, the data will be archived and displayed normalized to the 1.0 mm pathlength. The feature can be turned off using the UV/Vis tab in the Users Preferences module.

- **nm 1/abs1 and nm 2/abs2:** current values of the user-selectable wavelength cursors and corresponding absorbencies for a 1 mm pathlength. The wavelengths can be set by using the up/down arrows or typing in the desired wavelength. Note: The wavelength cursors will disappear if the default settings are changed but will reappear once a measurement is complete.

- **Normalize:** a user-selectable feature in this module. If selected, the software will automatically normalize the spectrum based on the lowest absorbance value in the range 400-700 nm.
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

9. Protein A280

Proteins, unlike nucleic acids, can exhibit considerable diversity. The A280 method is applicable to purified proteins exhibiting absorbance at 280 nm. It does not require generation of a standard curve and is ready for quantitation of protein samples at startup. This module displays the UV spectrum, measures the protein's absorbance at 280 nm (A280) and calculates the concentration (mg/ml). Like the Nucleic Acid module, it automatically switches to the 0.2 mm pathlength at very high concentrations of protein. Also analogous to the Nucleic Acid module, the Protein A280 module displays and records 10 mm (1 cm) equivalent data on the screen and in the archived data file.

Sample Volume Requirements

Some proteins are hydrophobic and others hydrophilic giving rise to variable surface tension properties in the sample to be measured. Additionally the presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter the surface tension resulting in difficulty forming adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 μ l sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form.

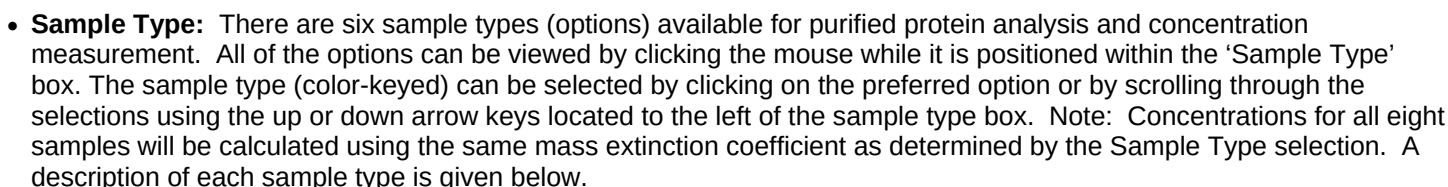
Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our [website](#).

Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer will accurately measure protein samples up to 100 mg/ml (BSA) without dilution. To do this, the instrument detects the high concentration and automatically utilizes the 0.2 mm pathlength to calculate the absorbance. The table below lists the concentration range and typical reproducibility for purified BSA measurements on the NanoDrop 8000:

Sample Type	Detection Limit	Approx. Upper Limit	Typical Reproducibility (minimum 96 replicates) (SD= mg/ml; CV= %)
Purified BSA	0.15 mg/ml	100 mg/ml	sample range 0.15-5 mg/ml: ± 0.15 mg/ml sample range >5 mg/ml: $\pm 2.5\%$

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for protein measurements as defined by the absorbance at 280 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.



- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding 10mm equivalent normalized absorbance at the respective wavelength absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.
- **A280 10-mm Path:** 10 mm-equivalent absorbance at 280 nm for the protein sample measured.
- **A260/280:** ratio of sample absorbance at 260 nm and 280 nm.
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

Spectrum Normalization

The baseline is automatically set to the absorbance value of the sample at 340 nm, which should be very nearly zero absorbance. All spectra are referenced off of this zero.

10. Proteins & Labels

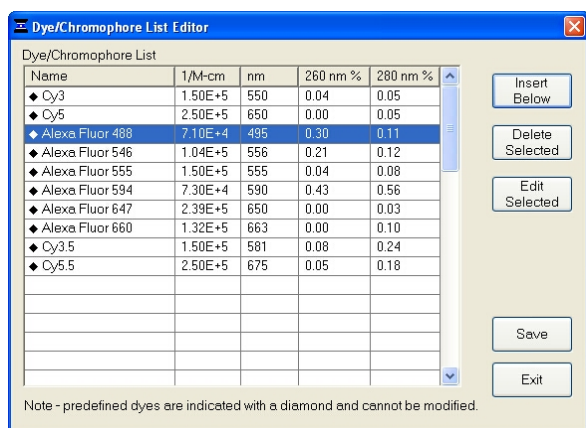
This software module can be used to determine protein concentration (280 nm) as well as fluorescent dye concentration (protein array conjugates), or to measure the purity of metalloproteins (such as hemoglobin) using wavelength ratios.

Fluorescent Dye Selection

There are currently ten fluorescent dyes that are hard-coded for use with the Proteins and Labels module (see table below). Users can also enter & save fluorescent dyes not coded within the NanoDrop 8000 software using the 'Dye/Chromophore Editor' button found in the main menu.

The respective absorbance wavelength, extinction coefficient, and 280 nm % corrections will be automatically utilized for measurement and concentration calculation. The default setting is Cy3. In addition to the fluorescent dyes available from the drop-down menu, an option on the main acquisition page entitled 'None' is also available. Selecting 'None' disables the respective calculations & numeric displays corresponding to a dye.

Note: Please refer to the dye manufacturer for the appropriate correction factors for user entered dyes.



Name	1/M-cm	nm	260 nm %	280 nm %
♦ Cy3	1.50E+5	550	0.04	0.05
♦ Cy5	2.50E+5	650	0.00	0.05
♦ Alexa Fluor 488	7.10E+4	495	0.30	0.11
♦ Alexa Fluor 546	1.04E+5	556	0.21	0.12
♦ Alexa Fluor 555	1.50E+5	555	0.04	0.08
♦ Alexa Fluor 594	7.30E+4	590	0.43	0.56
♦ Alexa Fluor 647	2.39E+5	650	0.00	0.03
♦ Alexa Fluor 660	1.32E+5	663	0.00	0.10
♦ Cy3.5	1.50E+5	581	0.08	0.24
♦ Cy5.5	2.50E+5	675	0.05	0.18

Note - predefined dyes are indicated with a diamond and cannot be modified.

Sample Volume Requirements

Additionally the presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter the surface tension resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 μ l sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form.

Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found at on our [website](#).

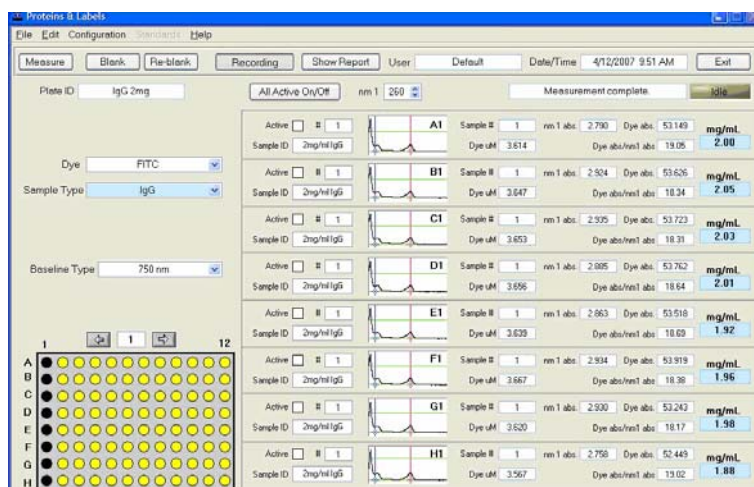
Measurement Concentration Range

The NanoDrop 8000 Spectrophotometer will accurately measure protein samples up to 20 mg/ml (BSA) without dilution. A table of concentration range and typical reproducibility is listed below:

Sample Type	Lower Detection Limit	Approx. Upper Limit	Typical Reproducibility (minimum 5 replicates) (SD= mg/ml; CV= %)
Purified BSA	0.15 mg/ml	20 mg/ml	sample range 0.15-5 mg/ml: ± 0.15 mg/ml sample range >10mg/ml: ± 2.5 %
Cy3	0.25 μ M	100 μ M	sample range 0.25-4 pmol/ul: ± 0.25 sample range >4.0 pmol/ul: ± 2.5 %

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be defined for the protein component in mg/ml as defined by the absorbance at 280 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features



- **Sample Type:** The same six sample types (options) listed in the Protein A280 section are available for purified Proteins & Labels analysis and concentration measurement. All of the options can be viewed by clicking the mouse while it is positioned within the sample type box. The sample type (color-keyed) can be selected by clicking on the preferred option or by scrolling through the selections using the up or down arrow keys located to the left of the sample type box. See the Protein A280 section for a detailed description of each sample type.
- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding 10mm equivalent normalized absorbance at the respective wavelength absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.
- **Dye abs:** normalized 10 mm equivalent absorbance of selected Dye.
- **Dye uM:** concentration of dye in sample based upon selected Dye's extinction coefficient. See the "Concentration Calculation (Beer's Law)" in the Appendix for more details on this calculation. Dyes can be selected using the scroll arrows or by highlighting the Dye box.
- **Dye abs/ nm 1:** ratio of the absorbance of the dye to the absorbance at the user-selected wavelength (nm 1).
- **mg/ml:** concentration of protein in the sample calculated using the absorbance at 280 nm minus the absorbance at 340 nm (i.e. normalized at 340 nm). Note: The user may elect whether or not to use the bichromatic normalization of the 280 nm absorbance value to the absorbance value at 340 nm. See the "Concentration Calculation (Beer's Law)" Appendix for more details on this calculation.
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

Baseline Type

This application module has two user-selectable “Baseline Type” options. The default setting is set to normalize the display spectrum at 750 nm. Alternatively, the 400-750 Slope Baseline Type will normalize the display at 750 nm and accommodate any linear baseline offset across the 400 to 750 nm range. The 280 nm absorbance value is normalized at 340 nm.

11. Protein BCA

The BCA (Bicinchoninic Acid) Protein Assay is an alternative method for determining protein concentration. It is often used for more dilute protein solutions and/or in the presence of components that also have significant UV (280 nm) absorbance. Unlike the Protein A280 method, the BCA Assay requires that a standard curve be generated before unknown protein concentrations can be determined. The resulting Cu-BCA chelate formed in the presence of protein is measured at its wavelength maximum of 562 nm and normalized at 750 nm. Pre-formulated reagents of BCA and CuSO₄, utilized in the assay, are available in kit form from numerous manufacturers. Follow the respective manufacturer's recommendations for all standards and samples (unknowns).

Sample Volume Requirements

The presence of surfactants or detergents in reagents can significantly alter the surface tension, resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form.

Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our [website](#).

Measurement Concentration Range

When using a 20:1 reagent to sample volume dilution, the BCA assay concentration range of detection is ~0.20 mg/ml to 8.0 mg/ml on the NanoDrop 8000. Using a 1:1 reagent to sample volume dilution the concentration range of detection is 0.01 mg/ml – 0.20 mg/ml.

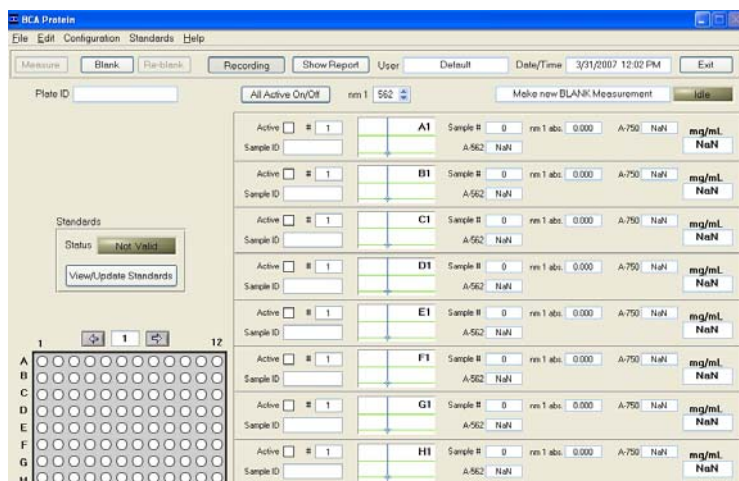
By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be set for protein (mg/ml) measured with this method at 562 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

BCA Assay Sample Preparation

Follow the manufacturer's protocol for the assay including recommended incubation times and temperature.

In addition to the kit reagents, protein standards (BSA) for generating a standard curve for the BCA method are often provided by the manufacturer. Use the respective standard (e.g., BSA) and dilutions that cover the analytical range (mg/ml) of interest. Note: Since the NanoDrop 8000 can measure higher protein concentrations than a cuvette based spectrophotometer, you may need to supply your own protein standards at higher concentrations than routinely provided by the manufacturer.

Unique Screen Features

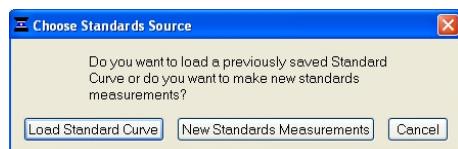


- **A 562nm:** the Cu-BCA complex's absorbance at 562 nm for the 1 mm pathlength.
- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.
- **mg/mL:** the concentration of the sample (unknown).
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

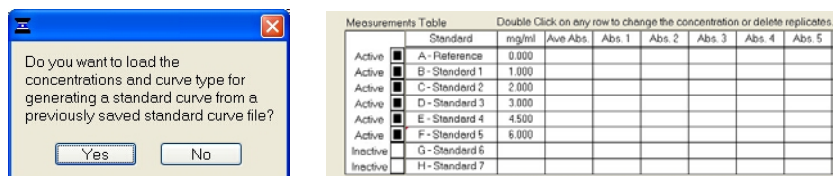
Making BCA Measurements

A standard curve is required every time the BCA assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines and generate fresh standard curves for each assay. Both single and multi-point standard curve generation are incorporated into the software. A standard curve can be developed using a reference (BCA reagent only – no protein) and a single replicate of one standard. The multi-point standard curve generator displays a maximum of 5 replicates for each of 7 different standards. There is no set order in which standards must be run.

The following box will appear after the module initialization is complete:

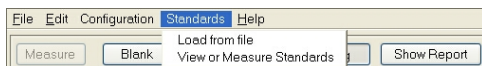


The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:



Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards. Selecting 'No' enables the user to enter new concentrations values for standards 1-7. The reference should remain set at 0.00.

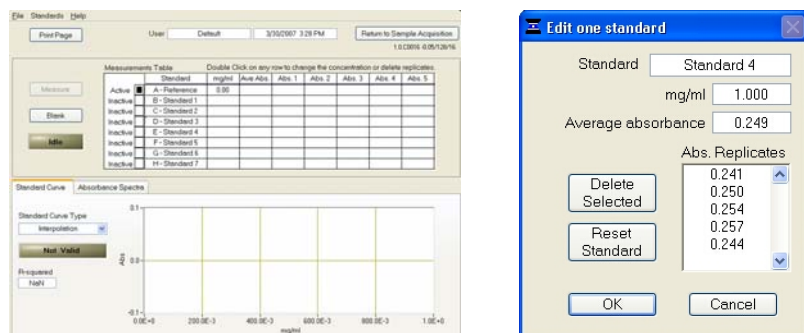
The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve.



Then follow the steps below to either generate or modify the curve as needed:

- **Enter the concentration for each standard.**

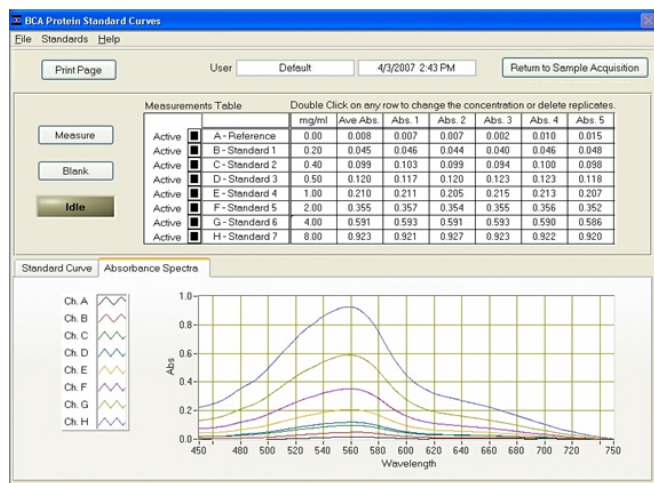
The user may either click on the Active/ Inactive box to the left of each standard or double click anywhere in the row of a particular standard to bring up the Edit Standard dialog box to enter in the concentrations for each standard. This box is also used to delete a single absorbance replicate value or reset the entire standard.



Alternatively, previously saved standard curves and standard curve concentration series may be loaded using the Standards menu bar drop-down options.

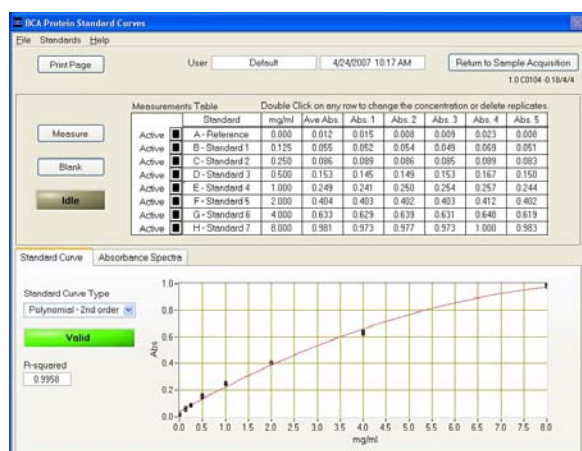
- **Measure Standards**

Up to 5 replicates of each standard will be displayed. The software will not allow measurement of samples until a minimum of either a reference and 1 standard or 2 standards – are measured. Polynomial curve fitting requires more standard points depending on the polynomial degree selected.

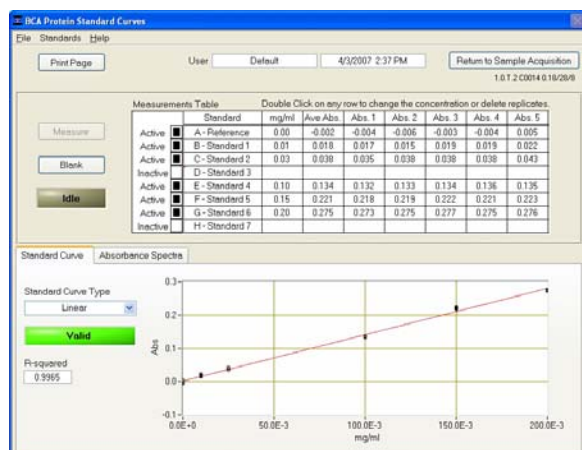


- **Measure Samples**

Once a minimum standard curve has been established, the standard curve indicator will change from gray (invalid) to green (valid), allowing the user to start measuring samples. The designation of "Valid" only indicates that the minimum requirement for a 2-point curve has been met. Sample concentrations are calculated by using linear interpolation (point-to-point) between the two standards flanking the unknown sample or by using polynomial fitting. Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the standard curve.



BCA Standard Curve
using a 20:1 reagent
to sample volume:
0.2 – 8.0 mg/ml



BCA Standard Curve
using a 1:1 reagent
to sample volume:
0.01– 0.20 mg/ml

Exiting the BCA Module

It is recommended that you process all of the unknowns to be assayed before exiting the BCA software module

12. Protein Lowry

The Modified Lowry Protein Assay is an alternative method for determining protein concentration based on the widely used and cited Lowry procedure for protein quantitation. Like the BCA and Bradford Assays, the Modified Lowry Assay requires that a standard curve be generated before unknown protein concentrations can be determined. The Modified Lowry procedure involves reaction of protein with cupric sulfate in an alkaline solution, resulting in the formation of tetradentate copper-protein complexes. The Folin-Ciocalteu Reagent is effectively reduced in proportion to the chelated copper-complexes resulting in a water-soluble blue product that is measured at 650 nm and normalized at 405 nm. Pre-formulated reagents, utilized in the assay, are available in kit form from numerous manufacturers. Follow the respective manufacturer's recommendations for all standards and samples (unknowns).

Sample Volume Requirements

The presence of surfactants or detergents in reagents can significantly alter the surface tension resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form. Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our [website](#).

Measurement Concentration Range

The Modified Lowry assay concentration range of detection is ~0.20 mg/ml to 8.0 mg/ml (BSA) on the NanoDrop 8000.

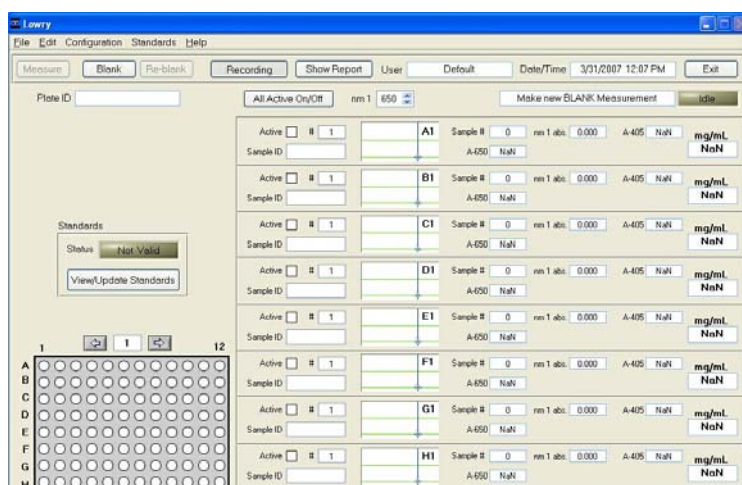
By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be defined for protein (mg/ml) measured with this method at 650 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Modified Lowry Assay Sample Preparation

Follow the manufacturer's protocol for the assay including recommended incubation times and temperature.

In addition to the kit reagents, protein standards (BSA) for generating a standard curve for the Modified Lowry method are often provided by the manufacturer. Use the respective standard (e.g., BSA) and dilutions that cover the analytical range (mg/ml) of interest. Note: Since the NanoDrop 8000 can measure higher protein concentrations than a cuvette based spectrophotometer, you may need to supply your own protein standards at higher concentrations than routinely provided by the manufacturer.

Unique Screen Features



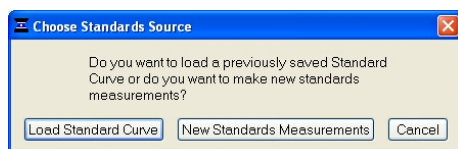
- **A 650nm:** the Cu-complex's absorbance at 650 nm for the 1 mm pathlength.
- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.
- **mg/mL:** the concentration of the sample (unknown).
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

Making Lowry Measurements

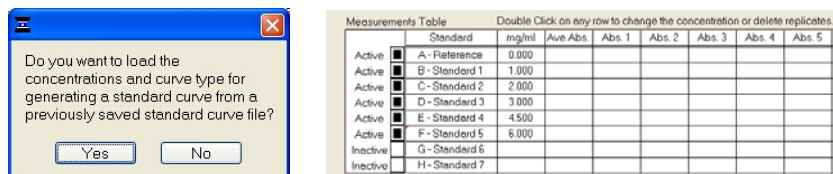
A standard curve is required every time the Modified Lowry assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines and generate fresh standard curves for each assay.

Both single and multi-point standard curve generation is incorporated into the software. A standard curve can be developed using a reference (Modified Lowry reagent only – no protein) and a single replicate of one standard. The multi-point standard curve generator displays a maximum of 5 replicates for each of 7 different standards. There is no set order in which standards must be run.

The following box will appear after the module initialization is complete:

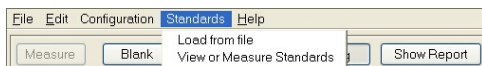


The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:



Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards. Selecting 'No' enables the user to enter new concentrations values for standards 1-7. The reference should remain set at 0.00.

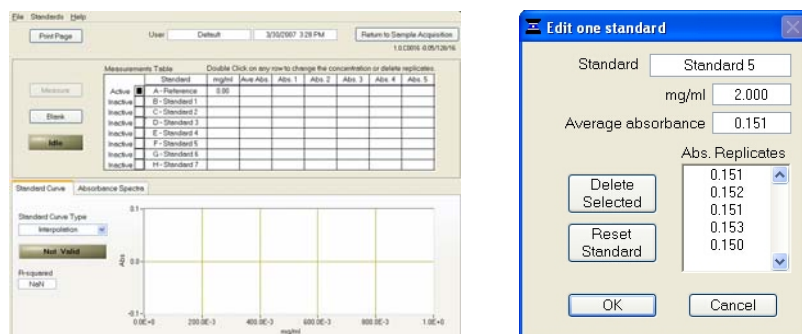
The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve.



Follow the steps below to either generate or modify the curve as needed:

- **Enter the concentration for each standard.**

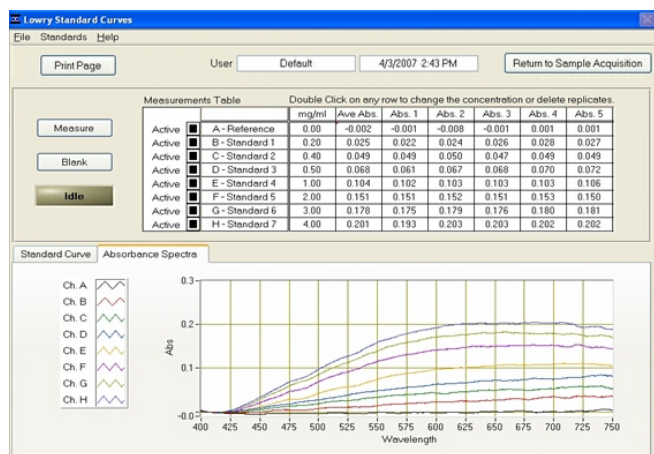
The user may either click on the Active/ Inactive box to the left of each standard or double click anywhere in the row of a particular standard to bring up the Edit Standard dialog box to enter in the concentrations for each standard. This box is also used to delete a single absorbance replicate value or reset the entire standard.



Alternatively, previously saved standard curves and standard curve concentration series may be loaded using the Standards menu bar drop-down options.

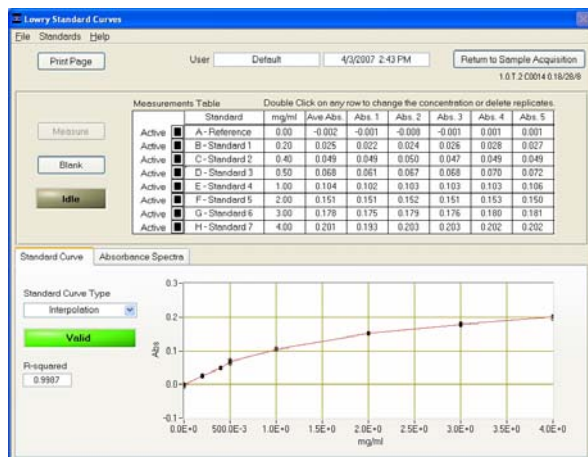
- **Measure Standards**

Up to 5 replicates of each standard can be measured. The software will not allow measurement of samples until a minimum of either a reference and 1 standard or 2 standards – are measured. Polynomial curve fitting requires more standard points depending on the polynomial degree selected.



- **Measure Samples**

Once a minimum standard curve has been established, the standard curve indicator will change from gray (invalid) to green (valid), allowing the user to start measuring samples. The designation of "Valid" only indicates that the minimum requirement for a 2-point curve has been met. Sample concentrations are calculated by using linear interpolation (point-to-point) between the two standards flanking the unknown sample or by using polynomial fitting. Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the standard curve.



Modified Lowry
Standard Curve: 0.2 –
4.0 mg/ml

Exiting the Lowry Module

It is recommended that you process all of the unknowns before exiting the Lowry software module.

13. Protein Bradford

The Bradford Assay is a commonly used method for determining protein concentration. It is often used for more dilute protein solutions where lower detection sensitivity is needed and/or in the presence of components that also have significant UV (280 nm) absorbance. Like the BCA method, the Bradford method requires that a standard curve be generated before unknown protein concentrations can be determined.

The Bradford uses the protein-induced Absorbance shift of Coomassie Blue dye to 595 nm as a measure of protein concentration. The bound protein-dye complex is measured at 595 nm and normalized at 750 nm. A single stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant in kit form is available from numerous manufacturers. Follow the respective manufacturer's recommendations for all standards and samples (unknowns).

Sample Volume Requirement

The presence of surfactants or detergents in reagents, such as the Bradford reagent, can significantly alter the surface tension resulting in difficulty forming and/or maintaining adequate columns for measurement. The column formation issue can be overcome without affecting the sample's absorbance by using a larger sample volume. **A 2 ul sample size is recommended for protein measurements.**

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Pedestal Reconditioning

Solutions and reagents containing surfactants may "un-condition" the measurement pedestal surfaces so that the liquid column does not form properly. If this occurs, "buff" the measurement pedestal surfaces by rubbing each measurement surface aggressively with a dry laboratory wipe 30-40 times. This will "re-condition" the surface allowing the liquid sample column to form. Alternatively, use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. Additional information about the PR-1 kit may be found on our [website](#).

Measurement Concentration Range

Using the regular Bradford assay the concentration range of detection is ~100 ug/ml up to several thousand micrograms/ml (ug/ml) on the NanoDrop 8000. The best linearity is in the 100ug/ml - 1000ug/ml range. The concentration range for the mini Bradford assay is 15 ug/ml to 125 ug/ml.

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum concentration limits can be defined for protein (mg/ml) measured with this method at 595 nm. These limits cannot be set as a default and must be defined each time the application module is opened. Protein measurements that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The sample concentrations will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

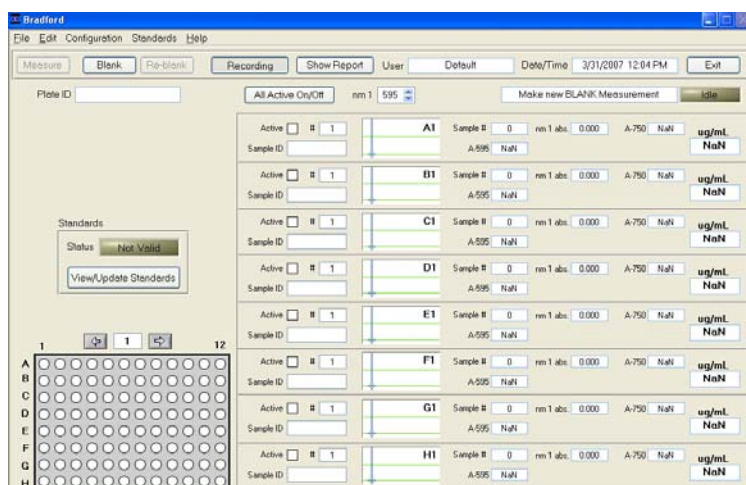
Coomassie dye-dye and Coomassie dye-protein aggregates are frequently encountered in Coomassie dye-based protein assays. With time, particulate can be observed, which can cause significant fluctuations in Absorbance readings. It is also important to note the total analyte (protein-dye) signal at 595nm is limited to ~ 0.150 A as a result of the 1.0mm pathlength of the instrument, the Bradford (Coomassie dye) reagent concentration, and the acidic pH. Making measurements in **triplicate** of standards and samples (unknowns) is good practice, particularly with the limited assay signal obtained with the Bradford Assay.

Bradford Assay Sample Preparation

Follow the manufacturer's protocol for the assay including recommended incubation times and temperature.

In addition to the kit reagents, protein standards (BSA) for generating a standard curve for the Bradford method are often provided by the manufacturer. Use the respective standard (e.g., BSA) and dilutions that cover the analytical range (mg/ml) of interest. Note: Since the NanoDrop 8000 can measure higher protein concentrations than a cuvette based spectrophotometer, you may need to supply your own protein standards at higher concentrations than routinely provided by the manufacturer.

Unique Screen Features

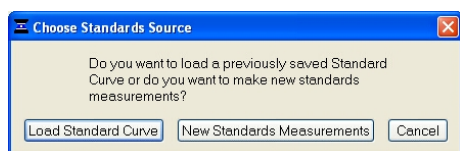


- **A 595nm:** protein-dye complex's absorbance at 595 nm at the 1mm pathlength.
- **nm 1 and nm 1 abs:** current value of the user-selectable wavelength cursor and corresponding absorbance value for a 1 mm pathlength. The wavelength can be set by using the up/down arrows or typing in the desired wavelength. Note: The user selected wavelength and absorbance are not utilized in any calculations.
- **ug/mL:** the concentration of the sample (unknown).
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

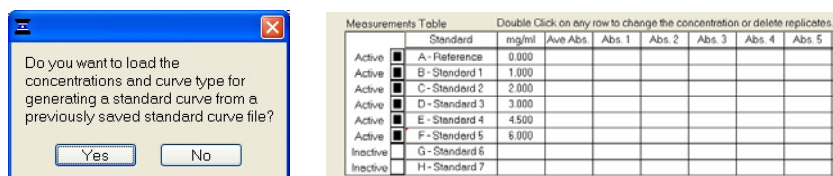
Making Bradford Protein Measurements

A standard curve is required every time the Bradford assay is run. Although curves can be saved and reloaded in the NanoDrop 8000 Spectrophotometer software, it is recommended that the user follow manufacturers' guidelines and generate fresh standard curves for each assay. Both single and multi-point standard curve generation is incorporated into the software. A standard curve can be developed using a reference (Bradford reagent only – no protein) and a single replicate of one standard. There is no set order in which standards must be run. The multi-point standard curve generator displays a maximum of 5 replicates for each of 7 different standards.

The following box will appear after the module initialization is complete:

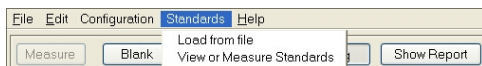


The user may load a previously saved standard curve or generate a new curve. Selecting the New Standards Measurements button will bring up the dialogue box on the left, below:



Clicking on the Yes button will allow the user to import just the Standard series without the respective measured values (See image on the right, above). This option is very useful when running a routine series of standards. Selecting 'No' enables the user to enter new concentrations values for standards 1-7. The reference should remain set at 0.00.

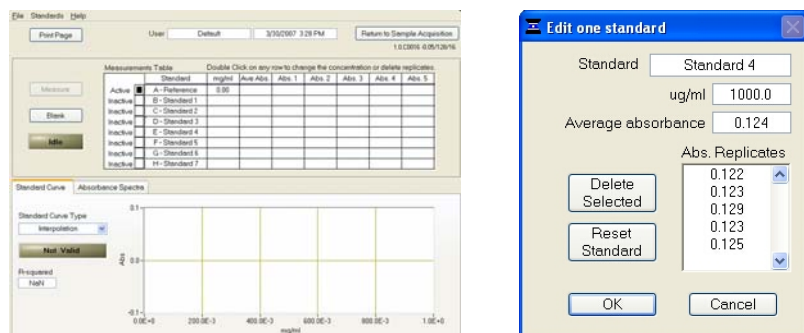
The Standards menu drop down may also be used to load a previously saved curve, generate a new standard curve, or view the current standard curve.



Then follow the steps below to either generate or modify the curve as needed:

- **Enter the concentration for each standard.**

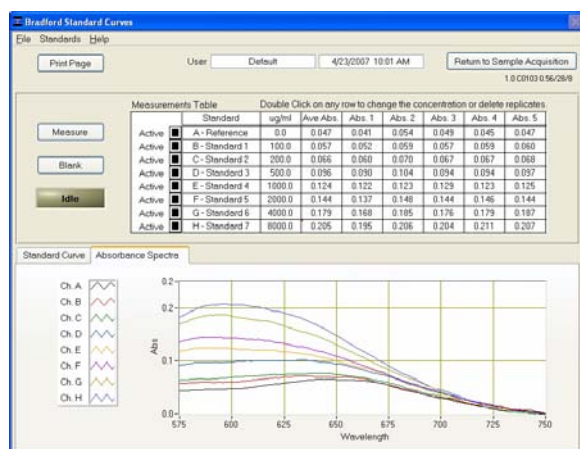
The user may either click on the Active/ Inactive box to the left of each standard or double click anywhere in the row of a particular standard to bring up the Edit Standard dialog box to enter in the concentrations for each standard. This box is also used to delete a single absorbance replicate value or reset the entire standard.



Alternatively, previously saved standard curves and standard curve concentration series may be loaded using the Standards menu bar drop-down options.

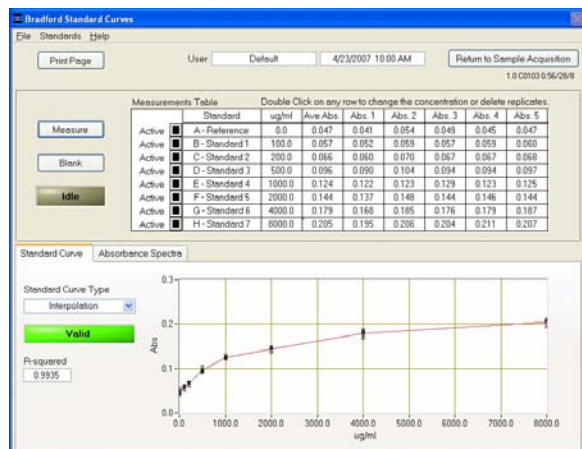
- **Measure Standards**

Up to 5 replicates of each standard can be measured. The software will not allow measurement of samples until a minimum of either a reference and 1 standard or 2 standards – are measured. Polynomial curve fitting requires more standard points depending on the polynomial degree selected.



- **Measure Samples**

Once a minimum standard curve has been established, the standard curve indicator will change from gray (invalid) to green (valid), allowing the user to start measuring samples. The designation of "Valid" only indicates that the minimum requirement for a 2-point curve has been met. Sample concentrations are calculated by using linear interpolation (point-to-point) between the two standards flanking the unknown sample or by using polynomial fitting. Note: In order to obtain a concentration value (mg/ml) the sample (unknown) must fall within the limits of the standard curve.



Regular Bradford curve using a 50:1 reagent to sample volume covers the range of 100-8000 ug/ml. Note the linear range is 100-1000 ug/ml

A Bradford assay using a 1:1 reagent to sample volume covers an approximate range of 15-100 ug/ml

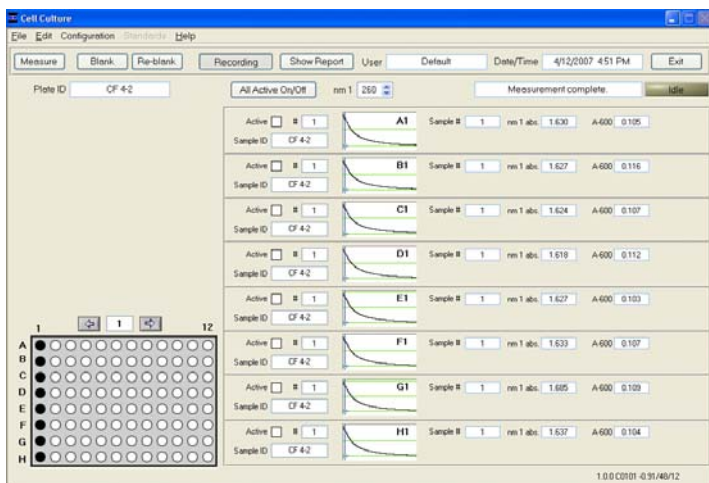
Exiting the Bradford Module

It is recommended that you process all of the unknowns to be assayed before exiting the Bradford software module

14. Cell Cultures

Using an absorbance spectrophotometer to monitor light scattered by non-absorbing suspended cells is common practice in life science laboratories. Such applications, more than any other, accentuate the differences between the optical systems of the numerous spectrophotometer designs.

Note: The most distinct difference between the NanoDrop 8000 Spectrophotometer “absorbance” values for cell microbial cultures and those observed using classical cuvette based systems will be attributable to the shorter pathlength (1 mm vs. 1 cm.) Values may not be exactly 10 fold different as readings are dependent on both the optics of a specific spectrophotometer as well as the cell type in suspension.



The Cell Cultures module displays the sample spectrum from 250 nm to 700 nm. The software will display the absorbance data for the frequently used wavelength for monitoring cell suspensions (600nm) in addition to displaying the value for a second wavelength of interest.

By selecting Measurement Limits from the configuration drop down menu, minimum and maximum absorbance limits can be set for the user selectable wavelength (cursor position). These limits cannot be set as a default and must be defined each time the application module is opened. Sample absorbances that are outside of the defined range will be indicated by a flashing light on the Sample Position Illuminator. The absorbances will also appear in red when the plate summary is displayed. Once the plate summary has been reviewed, samples of interest marked for repeat, and the window closed, the Sample Position Illuminator will stop flashing.

Unique Screen Features

- **600nm Absorbance:** current value of the absorbance at the $\lambda 1$ cursor with the baseline absorbance subtracted.
Note: The actual 1 mm absorbance is displayed.
- **nm 1:** current value of the user-selectable wavelength cursor and corresponding absorbance. The wavelength can be set by using the up/down arrows or typing in the desired wavelength.
- **Show Report:** formatted for 1000 samples although the buffer size can be modified.

Sample Size Requirements

Field experience has indicated that 1ul samples are sufficient to ensure accurate and reproducible results when measuring aqueous samples. However, if you are unsure about the surface tension properties of your sample or your pipettor accuracy, a 1.5-2 ul sample is recommended to ensure that the liquid sample column is formed and the light path is completely covered by sample.

Use an 8-channel pipettor when loading multiple samples to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Cell Suspension Concentrations

Due to its shorter pathlength, the NanoDrop 8000 can measure absorbances that are 10-fold higher than those measurable on a standard cuvette spectrophotometer. This makes it possible to directly monitor concentrated cell suspensions. Since the entire spectrum is displayed, diluted samples exhibiting very low 'Absorbance' at 600 nm can be monitored at lower wavelengths, for example 300 nm.

Sample Homogeneity

The user must be sure to homogeneously suspend the cells when sampling for "absorbance" measurements and read the sample immediately to avoid significant cell settling. Vigorous mixing may be required particularly when measuring concentrated samples

Decontamination of Measurement Pedestals

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. The metal fiber optic fittings are made from 303 stainless steel and are resistant to most common laboratory solvents (see "Solvent Compatibility" appendix.) A final cleaning of all surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do not use a squirt bottle to apply bleach or de-ionized water. Routine use of ethanol or isopropanol for cleaning is not recommended

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15. Archived Data and Data Viewer

Sample data from all application modules is automatically stored in archive files and can be opened by either the integrated Data Viewer software program or spreadsheet programs such as MS Excel.

Archive File Creation

Every time an application module is started, an application-specific archive file is created for the user that is logged in. All measurements made by the user (in that application module) for a given calendar day are stored in a single archive file. These files bear the name of the respective application module with the date appended. For example, an archived file entitled "Nucleic Acid 2007 03 21.nd8" corresponds to Nucleic Acid data from the software session that began on March 21, 2007. A unique file extension (.nd8) has been given to these files to enable automatic startup with the Data Viewer (see the description of Data Viewer later in this section).

The data may be edited and/or reformatted and stored under names of the user's choice. The spectrum can be re-plotted from the wavelength data if needed for further analysis.

Absorbance data shown in archive files are represented as they are displayed on the screen. For Nucleic Acids, Protein A280, and Protein and Labels application modules, data is stored based on a 1.0 cm (10.0 mm) path. For MicroArray, UV-Vis, BCA Protein, Bradford, Lowry and Cell Culture application modules the data is normalized to a 1.0 mm (0.1 cm) path.

For data from all modules, a column entitled 'Measurement Type' is included. For each measurement, this column will contain 'Measure', 'Blank', or 'Reblank'. If the value is 'Measure', then the values in that row are from a normal measurement that has utilized the stored blank value. If the value is 'Blank', it indicates that the measurement is the initial blank recorded. If the value is 'Reblank', it is the re-analysis of the previous measurement with a new blank.

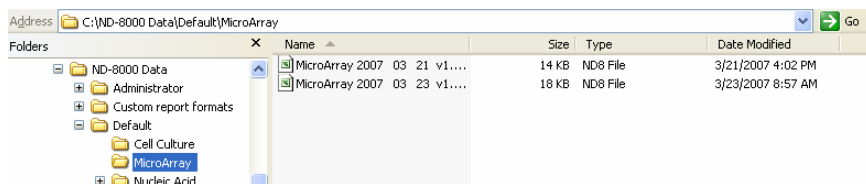
Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos	Cursor abs	340 raw	Measurement
6	Default	11/1/2005	8:38 AM	0	0	0	NaN	NaN	50	230	0	0	Blank
7	Default	11/1/2005	8:39 AM	3.51	0.07	0.03	2.42	2.12	50	230	0.033	0	Measure
8	Default	11/1/2005	8:39 AM	43.76	0.88	0.52	1.67	2.43	50	230	0.36	0.000	Measure
9	Default	11/1/2005	8:40 AM	393.73	7.87	4.27	1.85	2.35	50	230	3.353	0.000	Measure
10	Default	11/1/2005	9:41 AM	3704.14	74.08	39.84	1.86	2.24	50	230	33.075	-0.036	Measure

Data Storage Hierarchy

The hierarchy for archive files is as follows:

C:\ND-8000 Data → User name → Application Module (BCA Protein, Lowry, Bradford, Cell Culture, MicroArray, Nucleic Acid, Protein A-280, Proteins & Labels, UV-Vis).

All archived data files are stored within an application module folder that is within the User folder as shown below:



User-Defined Data File Export Location

In addition to the primary data storage, users may elect to export their data to an additional location. This option can be chosen under the Data Export Exporting tab in User Preferences on the Main Menu. Select the Automatic Data Export feature by enabling the On box. Select the data export destination using the icon next to the Data Export Folder dialog window. Save the alternative path by clicking on the 'Save and Exit' button before exiting the User Preferences window.

All data are written to the archive file immediately upon completion of the measurement. Inadvertent software or PC shutdowns should not affect the archive file.

Data Viewer

Data Viewer is a versatile data reporting software program incorporated into the operating software that offers the user the ability to customize report structures, import stored data and re-plot data from previously generated data. Using the Data Viewer is the most expedient method to review data. This feature may be accessed during measurement sessions from the Show Report function found within each method module. It may also be accessed from the Main Menu page. A NanoDrop 8000 Spectrophotometer does not need to be connected to the PC to use the Data Viewer module.

Data Viewer Features

The Data Viewer is composed of two or three pages in a tabular form consisting of Plots, Reports and Standard Curves (where utilized). The user may access any page by clicking on the tabs.

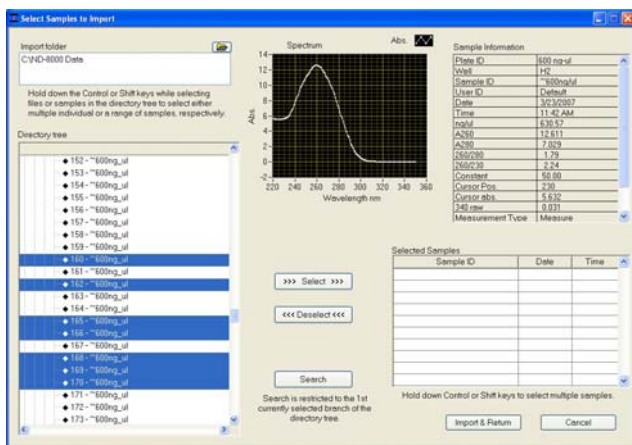
The software opens to the Report page whether accessed through the Main Menu or Show Report. Note: Recording rather than Start Report must be selected in order to access the Data Viewer via Show Report.

Tool Bar Features common to all three pages include:

- **File:** Allows the user to define the page set up for printing out the spectra, the report and the standard curve. This drop down also allows the user to save the window as a jpg.
- **Configuration:** Options controlled by this tool bar function include 'Auto Scale', 'Include graph in printout' and 'Include standards in printout'.
- **Data:** Includes options to import data, rename samples and delete sample data. Note: After deleting all samples, it is important to exit out of the Data Viewer module and re-enter if importing data for a different application type.
- **Reports:** This tool bar function allows the user to select columns of interest to be included in a report. See following section on Reports Page for details on additional drop down box options.
- **Print Window:** The current Plot, Report, or Standards screen may be printed by selecting 'Print Window' or 'CTL +P'.
- **Save Window:** Saves files as .JPGs.

Import Page

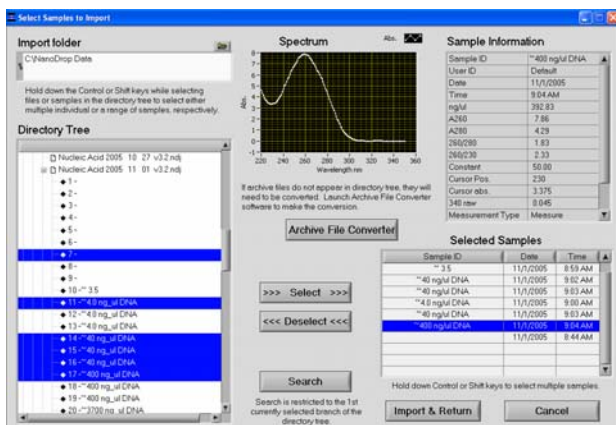
To select samples for viewing, select 'Import Samples' from the Data menu bar drop down options from either the Plots or Report pages within the Data Viewer software. This will bring up a new window with an Import Folder box and a Directory Tree as shown below.



Features include:

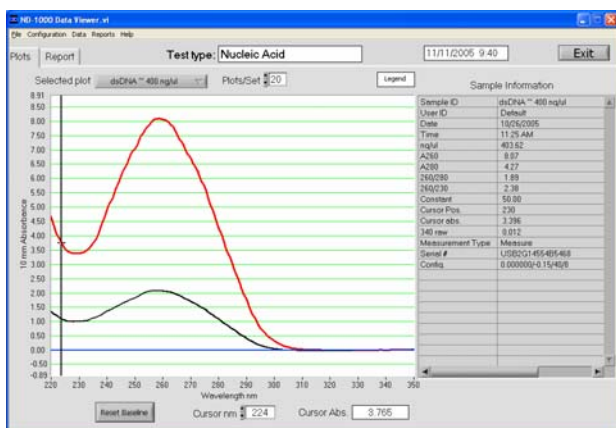
- **Import folder:** Used to select folder where data is to be imported from. Folder selection must be at the level of user or higher--may not select an application or method folder within a user in this activity box.

- **Directory tree:** Used to select specific data to be imported. Clicking on the square to the left of each file name will provide further detail to each level. Users may choose to select either individual samples within a file or the entire file. All import selections must be of the same application or method type.
- **Select or Deselect:** Used to move the highlighted sample choices to or from the Selected Samples box. Note: The software defaults to a buffer size of 1000 samples.
- **Search:** Function allows the user to locate specific data by searching through sample ID names.
- **Sample Information and Spectrum:** Are populated with the information associated with the most recently highlighted sample.
- **Import and Return:** Uses selected sample data to populate Plots and Reports windows. Note: Holding down the shift or control PC function keys will allow the user to select multiple samples and/or files for importing. The keys can also be used to deselect multiple samples. See example below:



Plots Page

The Plots page displays selected sample spectra.



Features include:

- **Test Type:** Auto fills in module name.
- **Date:** Auto fills in date and time of report.
- **Selected Plot:** There are two methods of selecting or highlighting individual sample data. The user may simply move the cursor over the plot of interest and click or use the 'Selected Plot' drop down box (which will also display the legend). The selected sample will show up as a bold plot line.

The Report page displays the data for selected samples in a table format. The user may modify column configurations for each method type and save multiple customized formats.

ND 8000 Data Viewer.vi

File Configuration Data Reports Help

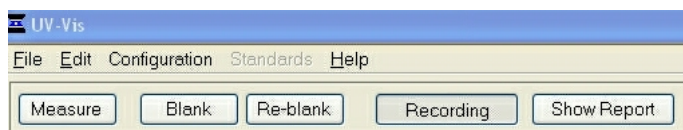
Plots | Report

Test type: Nucleic Acid 4/3/2007 3:05 PM Exit

Report Name: Report Full Mode: Ignore

Plate ID	Well	Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Conisitant	Cursor Pos.
600-ng/ul	H2	~600ng/ul	Default	3/23/2007	11:42 AM	630.57	12.611	7.023	1.79	2.24	50.00	230
600-ng/ul	A3	~600ng/ul	Default	3/23/2007	11:43 AM	607.95	12.159	6.623	1.84	2.24	50.00	230
600-ng/ul	B3	~600ng/ul	Default	3/23/2007	11:43 AM	595.13	11.903	6.534	1.82	2.25	50.00	230
600-ng/ul	F2	~600ng/ul	Default	3/23/2007	11:42 AM	601.92	12.038	6.709	1.79	2.24	50.00	230
600-ng/ul	E2	~600ng/ul	Default	3/23/2007	11:42 AM	603.78	12.076	6.713	1.80	2.25	50.00	230
600-ng/ul	H1	~600ng/ul	Default	3/23/2007	11:41 AM	610.59	12.212	6.790	1.80	2.23	50.00	230
600-ng/ul	B2	~600ng/ul	Default	3/23/2007	11:42 AM	552.05	11.841	6.395	1.85	2.32	50.00	230

All data is automatically archived. The user can log measurement results in an active report table as the data is accumulating by using the Start Report / Recording feature in the acquisition module. The default setting has the Recording feature activated for all modules. If 'Start Report' is displayed, the accumulating data will still be archived but will not be shown in the active report.

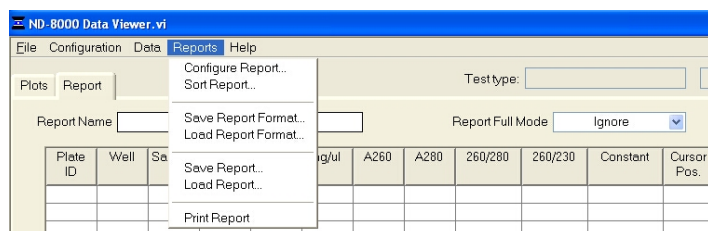


Selecting this button will bring up the Report page. Hitting the Exit button at the top right will exit back to the acquisition module. The data will continue to populate the report after exiting.

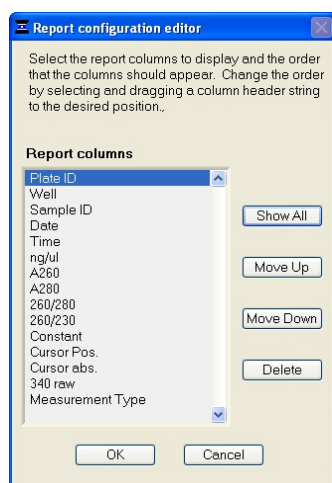
[illegible]

All data is stored in the archive file at c:\IND-8000 Data and may be exported to an alternate location. To open these reports, go to the C:\IND-8000 Data\Reports folder and right click on the file of interest.

Some key options useful for the Report page are accessible through the Report tool bar drop down.



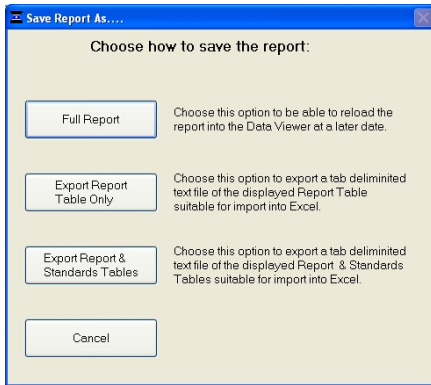
Choosing the **Configure Report** option brings up the following box:



Selecting 'OK' will return the user to the Report page displaying only the columns of interest.

Additional options include:

- **Sort:** Allows users to sort data by column (example-by date or sample name) and by either ascending or descending order.
- **Save Report Format:** Saves the current report format as an .nr8 file for retrieval and future use. To designate a saved report format as the default format, exit to the Main Menu, choose 'Users Preferences' and click 'Reports'. Use the "Select Default Report Format" to see the list of saved formats available for the specific method type.
- **Load Report Format:** Allows saved report formats to be loaded either before or after data is imported.
- **Print report:** Will print out only the Report page by default. Users may choose whether or not to print out the standards or plots pages by selecting these options under the Configuration drop down on the tool bar.
- **Save Report and Load Report:** There are several options for this feature as seen in the following window:



Using the Full Report option will allow the user to use the Data Viewer to reload the report at a later date. The saved report may be recalled using the pull down Load Report. If using the Load Report feature- the report will be displayed with the default column configuration. Note: Access the User Preferences module on the main menu to modify and save preferred default configurations. Reports are saved in an .nr8 format.

The other two options are meant for reports that are expected to be opened in Excel type spreadsheets. To open these reports, go to the *C:\IND-8000 Data\Reports* folder and right click on the file of interest.

Additional features of the Report page:

- **Method:** Automatically populated with data method type.
- **Date and Time:** Automatically populated when report is generated.
- **Report Name:** User defined designation for the current report
- **Report Full mode:** Drop down box defining options for managing reports .
The user may elect to 'Print', 'Save', or 'Print and Save' a report at any time by using the Report Full Mode drop down box shown below. The default setting of 1000 samples per report may be modified by highlighting the box and typing in the desired number. Choosing 'Ignore' from the drop-down will allow the user to include an unlimited number of samples in a report.
- **Max Report Size:** Default number is set at 1000.

Standards Page

The Standards page will display the actual reference standards applied to each particular sample at the time of measurement. Note: This page is only available for software modules utilizing a Standard Curve.

File Configuration Data Reports Help

Plots Report Standards

Test type:

Standards

Sample ID	Curve Type	Ref conc	Ref Abs	Std 1 conc	Std 1 Abs.	Std 2 conc	Std 2 Abs	Std 3 conc	Std 3 Abs	Std 4 conc
Reference	Interp	0.00	0.029	NaN	NaN	NaN	NaN	NaN	NaN	NaN
Standard 1	Interp	0.00	0.029	100.00	0.047	NaN	NaN	NaN	NaN	NaN
Standard 2	Interp	0.00	0.029	100.00	0.047	1000.00	0.099	NaN	NaN	NaN
Standard 3	Interp	0.00	0.029	100.00	0.047	1000.00	0.108	2000.00	0.073	NaN

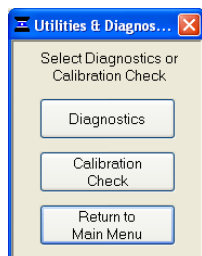
Opening Archived Data with Spreadsheet Programs

The archived files are in tab-delimited format and can be opened in Microsoft Excel or an equivalent spreadsheet program. To open these reports, go to the *C:\IND-8000 Data\Reports* folder and right click on the file of interest.

Note: Save and rename files before making any changes if opened with Excel type of programs to ensure that the original archived data is available for importing using the Data Viewer function

16. Calibration Check

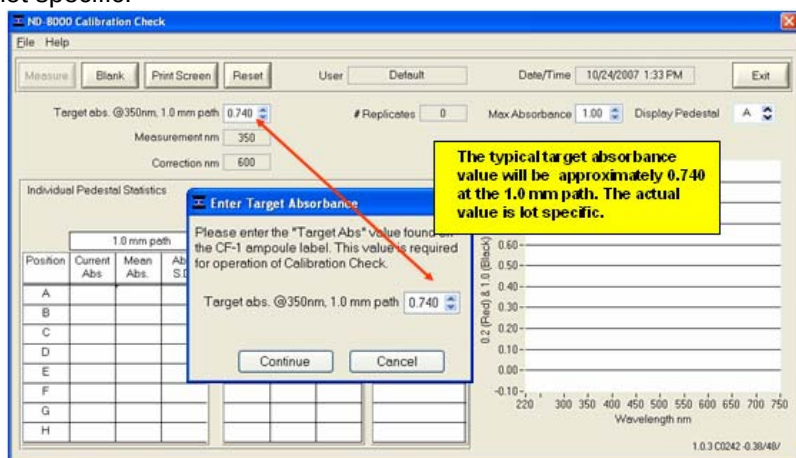
The Calibration Check is found within the Utilities and Diagnostics module and is accessed through the Main Menu. It is used to confirm that both pathlengths are within calibration specifications.



A CF-8 kit is required to run the calibration check procedure. The kit includes 8-well PCR strip tubes and an aqueous potassium dichromate ($K_2Cr_2O_7$) solution (CF-1) for use in confirming calibration of NanoDrop 8000 Spectrophotometers

Preparation

- Ensure the measurement pedestals are clean and that a 1.5 ul water sample “beads” up on each of the lower pedestals. Follow the cleaning and reconditioning steps below if the water flattens out and covers the pedestal.
 1. Apply 5 ul of dH₂O onto each bottom pedestal.
 2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2-3 minutes.
 3. Wipe away the water from each upper and lower pedestal with a clean lab wipe.
 4. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.
 5. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals.
 6. Wait 30 seconds for the PR-1 to dry.
 7. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed. Note: The black appearance of the removed residue is normal.
- Enter the Target Absorbance in the appropriate box labeled “Target absorbance @350 nm 1 mm pathlength”. The target absorbance is between 0.710 and 0.760, but the actual value can be found on the CF-1 ampoule label and is lot specific.



- Before opening an ampoule of CF-1 Calibration Fluid, shake vigorously to thoroughly mix solution. Ensure all liquid is collected in the bottom portion of the ampoule.
- Carefully break the neck of the ampoule to open the CF-1 calibration fluid and aliquot 60 ul into each of the 8 wells of the PCR strip.

NOTE: CF-1 is supplied in a single use vial and must be used within 30 minutes of opening the vial. Prolonged exposure to the environment may cause a significant concentration change.

Procedure

- Using an 8-channel pipettor, simultaneously add a 1.5uL aliquot of water to each pedestal position, lower the arm and click on the Blank button. At the end of the measurement cycle, use a lab wipe to remove the water aliquots.
- Using an 8-channel pipettor, simultaneously add a 1.5uL aliquot of CF-1 Fluid to the pedestal and click on the Measure button. At the end of the measurement cycle, use a lab wipe to remove the CF-1 aliquots from both the upper and lower pedestals.
- Repeat and measure a total of 5 separate sets of replicates.

Results

- After the 5th measurement, the Calibration Check Results will be displayed on- screen.
- When the instrument passes the check procedure, a pop up box will indicate that the instrument works within specifications and the results will be saved as a .JPG image at *C:\IND-8000 Data\Calib check*.
- To print a copy of the results for your records, click the "Print Screen" button. A .JPG of the final results is automatically archived on the hard drive at: *C:\IND-8000 Data\Calib check*. If the instrument needs to be recalibrated, contact your local distributor or [Technical Support](#) for assistance.

Additional information

Cleaning instructions, the calibration check procedure as well as a table of calibration check tolerances may be accessed via the menu bar Help drop-down feature.

17. Troubleshooting

Instrument Not Found



This error might appear upon software startup and usually indicates that either the power supply or the USB cable is not properly connected or the software is not loaded properly. To troubleshoot, do the following:

1. Check that the power supply is connected to the instrument. Confirm that the instrument is getting power by observing that light can be seen through the USB opening on the rear of the instrument.
2. Confirm that the USB cable is connected to both the PC and the instrument. Note: There are internal USB drivers in addition to the USB cable. When attaching the USB cable, please wait at least 30 seconds for the multiple USB devices to be installed and recognized before opening the software.
3. If the cable is connected properly but the Instrument recognition error persists, open the Windows Device Manager by either right clicking on the *My Computer* icon on the desk top or selecting *Start → My Computer (right click) → Manage → Device Manager → NanoDrop Devices*.
4. If an unknown device, a yellow exclamation point or a question mark appears next to one of three expected NanoDrop devices, manually uninstall the device by right clicking and selecting Uninstall from the options displayed.
5. Disconnect the power supply from the instrument first and then disconnect the USB cable from the NanoDrop 8000.
6. Reconnect the power supply, wait 5 seconds and then reconnect the USB cable. At this point you may or may not see the Found New Hardware Wizard. If the wizard appears, follow the prompts for automatic installation of the software. Windows XP SP2 operating system will ask to allow it to search the internet for the proper software as shown- Select 'No, not this time'.

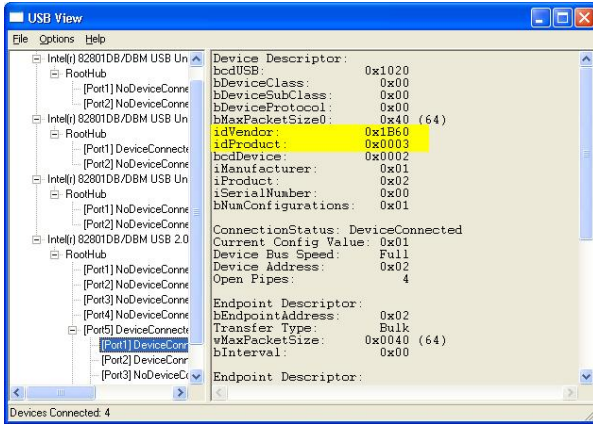


Intro Page: Windows XP- SP2



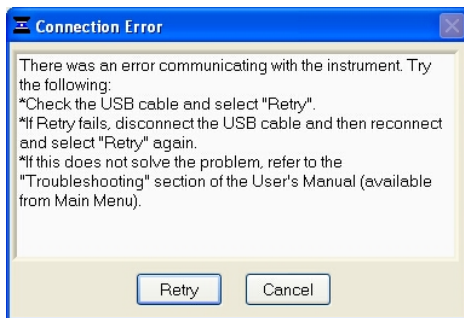
Other Windows Operating Systems

7. If the Found New Hardware Wizard appears, installation will require two cycles through the Wizard, once for the spectrometer and once for the peripheral control devices that are internal to the instrument. These devices need to install successfully for the NanoDrop 8000 to operate.
8. Restart the operating software. If the software works properly you are finished. If it does not operate properly, continue to step 9.
9. Close the operating software and open the USBView utility to confirm proper USB communication: *Start → Programs → NanoDrop → Utilities → USBView*. If USBView is not installed on your PC, you can download it from the 'Downloads' section of our [website](#).
10. Click on the 'Device Connected' (see example below). If more than one USB device is connected, view each of them. Three of the connected devices should display ID vendor numbers of 0x1B60 with ID products numbers of 0x0003, 0x0004 and 0x0005. If all three are present, the USB function of the instrument should be OK. If the "idVendor" and "idProduct" are different than indicated above, or if no USB device is present in the list, continue to step 11.



11. Install the instrument on another PC to rule out a faulty USB hub/port on the original PC. Run USBView on the 2nd PC. If the instrument is still not recognized, then the instrument may need service. Contact your local distributor or Technical Support for assistance.

Connection Error



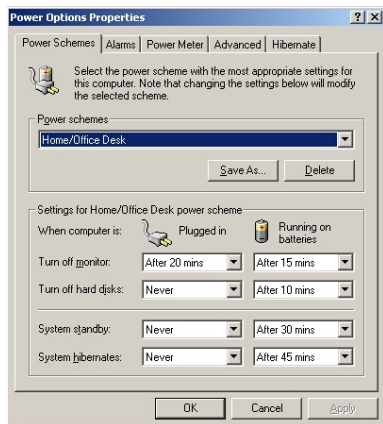
This error occurs whenever the USB connection is disrupted while operating a software module. Ensure that the USB cable is firmly inserted into the instrument and the computer then select 'Retry'. In most cases this will correct the problem. When attaching the USB cable, please wait at least 30 seconds for the USB devices and internal drivers to be installed and recognized.

Some additional possible causes for the error message and solutions are listed below:

Power management scheme on the PC:

If your PC is automatically going into standby or hibernate mode, the USB communication will be lost whenever it occurs and 'Retry' will NOT reconnect the instrument. If this occurs, the USB cable will need to be disconnected/reconnected before selecting 'Retry'.

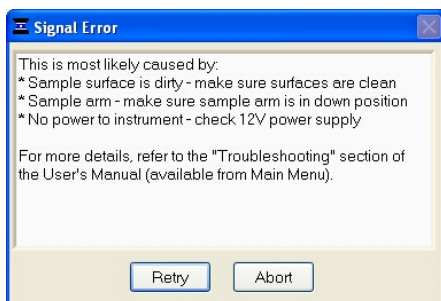
You can confirm that the power management settings are correct by opening the Power Options Properties page by choosing *Start* → *Control Panel* → *Power Options*. The 'System Standby' and 'System Hibernate' should be set to 'never' for the 'Plugged In' column.



Defective USB Port on PC

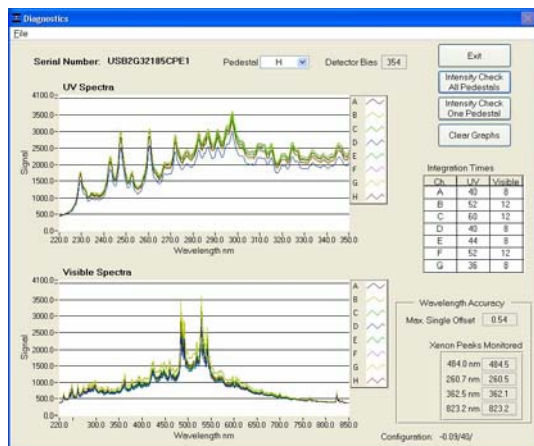
If your instrument operates properly most of the time, but the Connection Error appears intermittently, it could be caused by the USB port on the PC. If this occurs, install the software and operate on another PC. If the error does not occur on the second PC, it may be necessary to replace the USB card on the original PC.

Signal Error



This error occurs because no light or not enough light is reaching the detector. If the troubleshooting steps outlined in the message do not fix the problem, perform an Intensity Check:

- Open the Utilities and Diagnostics module from the main menu.
- With the sampling arm down, select OK to initialize the spectrometer and then select 'Intensity Check'. You will see two panels of 8 spectra each and a bias value greater than 65 as shown below. This indicates that the USB communication is normal, the power supply is operational and the flashlamp is functioning.



- If no spectra appear in the image, confirm that the power supply is firmly connected to the instrument and the plug is connected to a working outlet. Next, confirm that the power supply is operating properly. To do this, connect the leads of a volt-ohmmeter to the outlet of the supply. The voltage should be 12-20 Vdc, center positive.

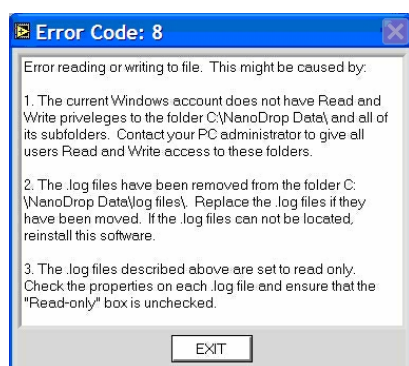
If none of the troubleshooting steps above solves the problem, contact your local distributor or [Technical Support](#) for assistance.

Error Code 8013



The software installation process installs two USB drivers. The above error message indicates that the motor UBS driver was not properly installed. Follow the instructions given above for the 'Instrument Not Found' error. When attaching the USB cable, please wait at least 30 seconds for the USB devices and internal drivers to be installed and recognized.

Error Code 8



This error code is most likely to occur if the user does not have read and write access to the folder *c:\ND-8000 Data* or one of its subfolders. See your PC administrator to make sure that all users of the operating software have the appropriate Windows access level.

Can't Find LabView RunTime Engine...

This error message likely means that one or more of the software components have been removed or corrupted. If this occurs, reinstall the Labview Runtime engine by selecting Start → Programs → NanoDrop → Utilities → Runtime Installer.

Report Format Loading Error



This error occurs when the user does not have "Write" access to one of the report format files located at *c:\WD-8000 Data\custom report formats* or the file has been moved from this folder. This error is similar to Error Code 8 (see above). Contact your PC administrator to give all users Read and Write access to this folder. Replace the files if they have been removed. If the file cannot be located, reinstall the software.

Other Software Error Messages

- **Source Error...**

This error indicates that there is insufficient light getting through to make good absorbance measurements. Check that the sampling arm is in the down position and the power is connected.

- **No Active Samples for Measurement...**

This error indicates that there are not any sample positions currently selected for measurement. The user may click either 'All Active' or select individual positions for the next measurement.

- **Error 8005**

This error occurs when trying to load a plate file that is not in a .txt format.

- **Error 9000**

This error occurs when the passwords.log file is missing or corrupt. Reinstall the operating software and "overwrite" the existing copy when prompted." A new copy of the passwords.log file should appear in the C:\ND-8000 Data\Log Files folder.

- **Error 9003**

This error indicates that the monitor resolution is below the 1024x768 required. Check the computer settings. Be sure that the Start menu tool bar is set to the bottom and not along the side.

- **Low Detector Bias...**

This occurs when the software has identified a problem with the detector. Contact your local distributor or [Technical Support](#) if you encounter this error.

- **Driver X Configuration Failed- You Must Manually Edit the Registry**

This error message (or others with similar wording) occurs when attempting to install the operating software on a computer running Windows 2000 or XP. It occurs because the user does not have the necessary authorization to install the software. Contact your system administrator if this occurs.

- **Insufficient Memory...**

This error message (or others with similar wording) occurs when attempting to install the operating software on a computer that does not have at least 100 MB of free hard disk space.

Liquid Column Breakage



This warning occurs when a possible problem with the column is detected. The software compares the long path and short path absorbances and issues a warning to the user if the short path is not 20% of the long path absorbance, within a tolerance. The most common explanation is that the column is not forming properly due to the pedestal being "unconditioned".

When a pedestal becomes unconditioned, sample droplets applied to the bottom pedestal will 'flatten-out' and cover the entire pedestal surface rather than 'bead up'. Buffers containing detergents and various other reagents may cause the pedestal surfaces to become unconditioned. We have noted that routine use of the Bradford reagent may result in difficulty forming columns with 1 ul samples.

Reconditioning

Use the NanoDrop Pedestal Reconditioning Compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.,
2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals.
3. Wait 30 seconds for the PR-1 to dry.
4. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed. Note: The black appearance of the removed residue is normal.

The reconditioning process is complete once the laboratory wipe shows no more black residue. To check the effectiveness of the reconditioning, load a 1 ul aliquot of dH₂O onto the lower measurement pedestals and visually verify that the water “beads” up.

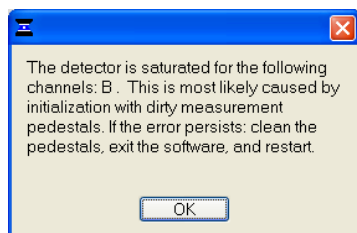
As an alternative to using the PR-1 Kit, the pedestals may be reconditioned as follows:

- Fold a clean dry lab wipe over several times to increase its thickness.
- Press the lab wipe firmly down on the lower pedestal and “buff/rub” very aggressively at least 50 times - the lab wipe will rip during this procedure and will have to be refolded several times throughout the procedure. The upper pedestal may also be buffed but care should be taken not to put too much force on the upper arm.

To check the effectiveness of the reconditioning, load a 1 ul aliquot of dH₂O onto the lower measurement pedestals and visually verify that the water “beads” up.

If the warning persists and the user visually confirms that the liquid column is forming, contact your local distributor or [Technical Support](#) for assistance.

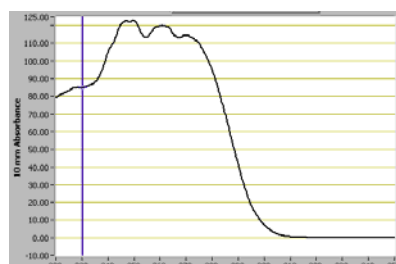
Saturated Detector



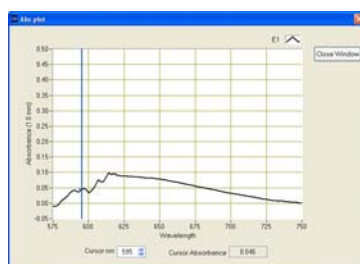
This error message can occur when the software calculates high integration times for particular positions during initialization. This is most likely due to the presence of an air bubble or a dried sample left on the measurement surface. Cleaning both the top and bottom pedestals with de-ionized water and exiting out of the software module to the main menu should alleviate the problem. It is not necessary to close the software completely as each module is re-initialized when it is opened. If the error persists, contact your local distributor or [Technical Support](#) for assistance.

Unusual Spectrum

A sample that exhibits jagged “cuts” out of the spectrum, but an otherwise normal shape, may be the result of detector saturation. This can be caused by the software selecting too high of an integration time due to a dirty sample pedestal upon startup. Try cleaning lower and upper sample pedestals thoroughly and restarting the software. For reference, examples of spectra generated with a saturated detector are shown below.



Detector saturation- nucleic acid measurement



Detector saturation- Bradford measurement

A spectrum that is very “un-smooth” or “ragged” can be caused by insufficient light intensity reaching the spectrometer. If you suspect that this is occurring, contact your local distributor or [Technical Support](#) for assistance.

Sample Accuracy and Reproducibility

If you are obtaining results that seem inaccurate or not reproducible, it could be the result of sample or aliquot non-homogeneity or liquid column breakage. It may be helpful to try the following to ensure representative results:

- **Make sure the sample surfaces are clean before starting the software module**

A dirty sample pedestal on startup can cause erroneous absorbance readings (even negative values) and signal saturation. It is always a good practice to clean the sample surfaces with de-ionized water to remove any dried sample that might be present. Note: Do not use a squirt bottle to apply de-ionized water.

- **Use a 1.5-2 ul sample size**

Very strange results can occur when the liquid sample column is not completely formed during a measurement. While making a measurement, visually confirm that the liquid column is formed. If necessary, try 1.5-2 ul samples to ensure the column is formed. Also, proteins and solutions containing surfactants are known to “un-condition” the measurement pedestal surfaces so that the liquid column does not form. Use the NanoDrop Pedestal Reconditioning Compound PR-1 as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement. See **Column Breakage** in this section for further details.

- **Heat DNA samples to 55 °C and vortex before measurement**

Due to the small volumes required by the NanoDrop 8000, it is extremely important to ensure that the sample being measured is homogeneous. Field experience has shown that samples containing large molecules such as genomic or lambda DNA are particularly susceptible to this phenomenon. Note: Larger volumes used by cuvette-based spectrophotometers will minimize or mask the effect of sample non-homogeneity.

- **Perform a Blanking Cycle**

This will confirm that the instrument is working well and that any sample carryover from previous measurements is not a concern. To run a blanking cycle, perform the following:

1. Open the application software module.
2. Load an aliquot of the blank (the same buffer, or solvent the unknown samples are in) onto each of the lower measurement pedestals and then lower the sampling arm into the ‘down’ position.
3. Click on the Blank button. When the measurement is complete, wipe off the buffer from all pedestals.
4. Select “All Active On” and analyze a fresh aliquot of the blanking solution on all pedestals using the ‘Measure’ button (F1). The result should be 8 spectra with relatively flat baselines near zero.
5. Wipe the blank from both measurement pedestal surfaces with a laboratory wipe and repeat the process until the spectrum varies no more than 0.005 A (1mm path).
6. Reload the plate list file before measuring samples if necessary.

- **Confirm that reference (blank) solution and solvent are the same material**

Buffers often absorb in the UV range and therefore it is critical to blank the instrument with exactly the same material that the sample is suspended in.

- **Confirm that your sample is not too dilute**

Measuring samples at or near the detection limit will result in measurements that can vary a significant amount. Refer to the table of concentration ranges provided within the respective application module section of the manual for lower detection limits.

- **Confirm instrument accuracy with CF-1**

CF-1 is a concentrated potassium dichromate calibration standard available from Thermo Fisher Scientific and its distributors. It is a good practice to check the instrument’s performance every six months with a fresh vial of CF-1.

260/280 Ratio

Many researchers encounter a consistent 260/280 ratio change when switching from a standard cuvette spectrophotometer to the NanoDrop 8000 Spectrophotometer. The three main causes for this are listed below:

• Change in sample acidity

Small changes in solution pH will cause the 260:280 to vary^{**}. Acidic solutions will under-represent the 260:280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3. If comparing the NanoDrop 8000 Spectrophotometer to other spectrophotometers, it is important to ensure that the pH and ionic strength of an undiluted sample measured on the NanoDrop 8000 is at the same as the diluted sample measured on the second spectrophotometer.

^{**} William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity: *BioTechniques* 22:474-481 (March 1997)

• Wavelength accuracy of the spectrophotometers

Although the absorbance of a nucleic acid at 260nm is generally on a plateau, the absorbance curve at 280nm is quite steeply sloped. A slight shift in wavelength accuracy will have a large effect on 260:280 ratios. For example, a +/- 1 nm shift in wavelength accuracy will result in a +/- 0.2 change in the 260:280 ratio. Since many spectrophotometers claim a 1 nm accuracy specification, it is possible to see as much as a 0.4 difference in the 260:280 ratio when measuring the same nucleic acid sample on two spectrophotometers that are both within wavelength accuracy specification.

• Nucleotide mix in your sample

The five nucleotides that comprise DNA and RNA exhibit widely varying 260:280 ratios^{***}. The following represent the 260:280 ratios estimated for each nucleotide if measured independently:

Guanine: 1.15
Adenine: 4.50
Cytosine: 1.51
Uracil: 4.00
Thymine: 1.47

The resultant 260:280 ratio for the nucleic acid being studied will be approximately equal to the weighted average of the 260:280 ratios for the four nucleotides present. It is important to note that the generally accepted ratios of 1.8 and 2.0 for DNA and RNA are “rules of thumb”. The actual ratio will depend on the composition of the nucleic acid. Note: RNA will typically have a higher 260:280 ratio due to the higher ratio of Uracil compared to that of Thymine.

^{***} Leninger, A. L. *Biochemistry*, 2nd ed., Worth Publishers, New York, 1975

Technical Support

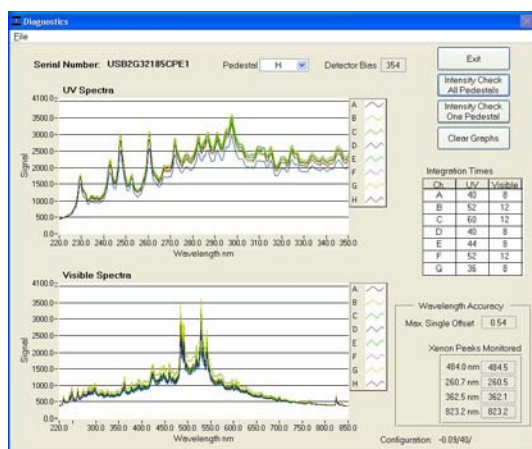
If, after referring to the above troubleshooting tips, you are unable to resolve your problem, please contact your local distributor or [Technical Support](#) for assistance. The following information will be very helpful:

• Serial Number of the instrument

The number can be found on the bottom of the unit.

• JPG image of Utilities and Diagnostics module

To get this, open this module and select OK to initialize the module. Select Intensity Check. Once the spectrum has been created, choose File → Save Window as shown below. Save to your hard drive and email as an attachment to your local distributor or to [Technical Support](#).



- **Application Module Screen Captures**

Screen captures of the actual spectrum as seen on your PC are of great use in diagnosing problems. Making a screen capture is quite easy. When in an application module, press Alt+Print Screen. This copies the highlighted screen window to the PC's clipboard. Next, paste this screen capture into MS Word, MS Paint (this program usually comes standard with the PC and can usually be found in the Start → Accessories menu), or other graphics programs. Save this as a .jpg or .doc file and send as email attachment to your local distributor or to Technical Support.

- **Data Archive Files**

If you have questions about your data, please send the archive file containing the suspect data as an email attachment to your local distributor or to Technical Support. The archived file can be found at *C:\IND-8000 Data → User name → Application Module* (BCA Protein, Bradford, Cell Culture, Protein Lowry, Proteins and Labels, MicroArray, Nucleic Acid, Protein A-280, UV-Vis)

18. Maintenance and Warranty

Cleaning

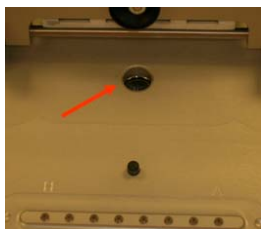
The primary maintenance requirement of the NanoDrop 8000 Spectrophotometer is to keep the measurement pedestal surfaces clean. Upon completion of each sample measurement, wipe the sample from the upper and lower pedestals to prevent sample carryover and avoid residue buildup. A final cleaning of all surfaces with de-ionized water is also recommended after the user's last measurement. Note: Do not use a squirt bottle to apply bleach or de-ionized water.

1. Apply 5 ul of dH₂O onto each bottom pedestal.
2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2-3 minutes.
3. Wipe away the water from each upper and lower pedestal with a clean lab wipe.

Note: Typically dH₂O is sufficient for removal of samples that have dried on the optical pedestals. There are a few cases (i.e. dried proteins) that may require a more rigorous cleaning protocol. For these cases, we recommend that 0.5M HCl with 5 ul of dH₂O to remove any residual HCl.

Cleaning of Light Source Window

The Xenon flash lamp window must be kept free of debris and obstruction. The window may be cleaned with a water-dampened laboratory wipe. Note: Do **not** use organic solvents such as acetone to clean the window.



Calibration

Pathlength (Accuracy) Calibration Check

It is good practice to check the calibration every six months using the CF-8 Calibration Fluid Kit. This will verify the pathlength accuracy of the instrument.

Wavelength

Each time the software is started, the wavelengths are auto-calibrated based on known peaks in the xenon lamp spectra. No calibration is required by the user.

Parts That Require Replacement

In general, the xenon flash lamp is the only part that will need to be replaced. The lamp has a lifespan of at least 30,000 measurements. When the flash lamp fails, the light output will become very erratic or stop altogether. Contact [Technical Support](#) or your local distributor if you suspect your lamp may need replacing.

Warranty

All NanoDrop spectrophotometers and accessories manufactured by Thermo Fisher Scientific are warranted against manufacturing defects in parts and labor for a period of one year. Preventive Maintenance as well as additional one, two, and three year warranty extensions are available. Additional information about the various plans may be found on our [website](#).

19. Appendices

Instrument Specifications

- Sample Size: 1microliter
- Sample Number: up to 8
- Path Length: 1 mm (with auto-ranging to 0.2 mm)
- Light Source: Xenon flash lamp
- Detector Type: 2048-element linear silicon CCD array
- Wavelength Range: 220-750 nm
- Wavelength Accuracy: 1 nm
- Wavelength Resolution: 3 nm (FWHM at Hg 546 nm)
- Absorbance Precision: 0.003 absorbance (1mm path)
- Absorbance Accuracy: 2% (at 0.76 absorbance at 257 nm)
- Absorbance Range: 0.02-75 (10 mm equivalent absorbance)
- Detection Limit: 2 ng/microliter (dsDNA)
- Maximum Concentration: 3700 ng/microliter (dsDNA)
- Measurement Cycle Time: 20 seconds
- Dimensions (footprint): 24 x 32 cm
- Weight: 3.5 kg
- Sample Pedestals Material of Construction: 303 stainless steel and quartz fiber
- Operating Voltage: 12 Vdc
- Operating Power Consumption: 30 W
- Standby Power Consumption: 3 W
- UL/CSA and CE approval: all units
- Included in system: software, compatible with Windows 2000 or XP
Vista has also been tested successfully with the software.

Blanking and Absorbance Calculations

When the NanoDrop 8000 Spectrophotometer is “blanked”, a spectrum is taken of a reference material (blank) and stored in memory as an array of light intensities by wavelength. When a measurement of a sample is taken, the intensity of light that has transmitted through the sample is recorded. The sample intensities along with the blank intensities are used to calculate the sample absorbance according to the following equation:

$$\text{Absorbance} = -\log (\text{Intensity}_{\text{sample}}/\text{Intensity}_{\text{blank}})$$

Thus, the measured light intensity of both the sample and of the blank are required to calculate the absorbance at a given wavelength.

Concentration Calculation (Beer's Law)

General

The Beer-Lambert equation is used to correlate the calculated absorbance with concentration:

$$A = E * b * c$$

Where **A** is the absorbance represented in absorbance units (A), **E** is the wavelength-dependent molar absorptivity coefficient (or extinction coefficient) with units of liter/mol-cm, **b** is the path length in cm, and **c** is the analyte concentration in moles/liter or molarity (M).

Fluorescent Dyes (MicroArray Measurement)

The software uses the general form of the Beer-Lambert equation to calculate fluorescent dye concentrations in the MicroArray module. The table of extinction coefficients for each dye is below:

Dye Type	Extinction Coefficient (liter/mol-cm)	Measurement Wavelength (nm)
Cy3	150000	550
Cy5	250000	650
Alexa Fluor 488	71000	495
Alexa Fluor 546	104000	556

Alexa Fluor 555	150000	555
Alexa Fluor 594	73000	590
Alexa Fluor 647	239000	650
Alexa Fluor 660	132000	663
Cy3.5	150000	581
Cy5.5	250000	675

Nucleic Acids

For nucleic acid quantification, the Beer-Lambert equation is modified to use an extinction coefficient with units of ng-cm/ml. Using this extinction coefficient gives a manipulated equation:

$$c = (A * e)/b$$

Where c is the nucleic acid concentration in ng/microliter, A is the absorbance in AU, e is the wavelength-dependent extinction coefficient in ng-cm/microliter and b is the path length in cm.

The generally accepted extinction coefficients for nucleic acids are:

- Double-stranded DNA: 50 ng-cm/ul
- Single-stranded DNA: 33 ng-cm/ul
- RNA: 40 ng-cm/ul

For the NanoDrop 8000 Spectrophotometer, path lengths of 1.0 mm and 0.2 mm are used compared to a standard spectrophotometer using a 10.0 mm path. Thus, the NanoDrop 8000 Spectrophotometer is capable of measuring samples that are 50 times more concentrated than can be measured in a standard spectrophotometer.

Note: Absorbance data shown in archive files are represented as displayed on the software screen. For Nucleic Acid, Protein A280 and Proteins and Labels modules, data are normalized to a 1.0 cm (10.0 mm) path. For MicroArray, UV-Vis, Protein BCA, Protein Bradford, Protein Lowry and Cell Culture modules the data are normalized to a 0.1 cm (1.0 mm) path.

Solvent Compatibility

The NanoDrop 8000 Spectrophotometer is compatible with most solvents typically used in life science laboratories. These include: methanol, ethanol, n-propanol, isopropanol, butanol, acetone, ether, chloroform, carbon tetrachloride, DMSO, DMF, acetonitrile, THF, toluene, hexane, benzene, sodium hydroxide, sodium hypochlorite (bleach), dilute HCl, dilute HNO₃, dilute acetic acid.

All forms of Hydrofluoric Acid (HF) are incompatible, as the fluoride ion will dissolve the quartz fiber optic cable.

Decontamination of Measurement & Optical Surfaces

If decontamination is necessary, a sanitizing solution, such as a 0.5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solution – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. The metal fiber optic fittings are made from 303 stainless steel and are resistant to most common laboratory solvents (see “Solvent Compatibility” appendix). A final cleaning of all surfaces with de-ionized water is also recommended after the user’s last measurement. Note: Do **not** use a squirt bottle to apply bleach or de-ionized water.